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MYCOTAXON

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REVIEWERS — VOLUME ONE HUNDRED THIRTY-ONE (4)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this issue.

Najam ul Sehar Afshan
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VIII ... MYCOTAXON 131(4)

PUBLICATION DATE FOR VOLUME ONE HUNDRED THIRTY-ONE (3)

MYCOTAXON *for* JULY–SEPTEMBER 2016 (I–XII + 491–734)

was issued on October 18, 2016

FROM THE EDITOR-IN-CHIEF

RICHARD P. KORF (1925–2016)—Our man in Ithaca was just too famous! When assembling materials for MYCOTAXON's special issue (scheduled appropriately as the final one in 2016), I encountered memorials, awards, over 400 manuscript titles eponymous names, biographies including a surprisingly complete Wikipedia entry plus three already published obituaries (not to mention plus Facebook blogs and tweets too numerous to count)—most accompanied by full color photos capturing our favorite curmudgeon's inherent greatness. How could his and Grégoire Hennebert's precious literary progeny possibly compete?

We can't. Fortunately this is not a competition (which Dick, in his own inimitable fashion, would have won hands down) but a chance to honor a man with panache and personality seemingly born to greatness. Thus we present photographs, long and short vignettes, and quips that we hope will bring smiles in the wake of a mycological giant who left us too soon.

THE EDITORIAL FINGER WRITES, AND HAVING WRIT – LIVES ON*—After flirting with using a black cover for this issue dedicated to Dick followed by a vain attempt to find a metallic gold that was bright instead of dull, we settled on the yellow-orange color selected last January for 2016's fourth issue. It was Noni, Dick's daughter, who realized that her dad's celebration issue would display the identical color he selected in 1973 for the first volume of MYCOTAXON. How pleased (and amused) Dick would have been at the coincidence.

WHAT DO WE DO WITH 'INVALID' HIGHER LEVEL TAXA? The MYCOTAXON dictum that all formal taxonomic names be italicized from subspecies to kingdom level sometimes causes problems. There was a fair amount of editorial hand wringing in December as to whether to distinguish the formal taxonomic subfamilies Xylarioideae and Hypoxyloideae from phylogenetic clades by using italics. As the two subfamilies are nomenclaturally invalid (Dennis proposed them in 1981 without the then-required Latin diagnoses), we decided to present them in roman font in this issue's Lee & al. (pp. 749–764) and Johnston & al. (pp. 765–771).

METABOLICALLY INACTIVE CULTURES—Although it seems that the INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI, AND PLANTS was only just revised, during July 23–29, 2017, the 19th International Botanical Congress will ratify in China a new "Shenzhen Code" to replace 2011's Melbourne Code. As the current code's Rec. 8B.3 ("When a culture is designated as a type, the status of the culture should be indicated, including the phrase 'permanently preserved in a metabolically inactive state' or an equivalent") is expected to become a requirement, MYCOTAXON now expects authors who designate cultures as types of new taxa to cite their inactive metabolic status in their type paragraphs.

*Adapted from Edward Fitzgerald's translation of the Rubáiyát of Omar Khayyám:
 "The Moving Finger writes; and, having writ, / Moves on: nor all thy Piety nor Wit/
 Shall lure it back to cancel half a Line, / Nor all thy Tears wash out a Word of it."

SPEEDY PUBLICATION, ACCURACY, AND AUTHORS—Of late, we have received far too many carelessly prepared submissions. Although we all too aware of the current delays our authors experience between accession and final nomenclatural approval, we nonetheless stress once again that all text and illustrations submitted to MYCOTAXON MUST be error-free. Manuscripts with unacceptable formatting, incoherent text, and spelling and grammatical errors will be returned to authors, with the returned revisions placed at the end of the editorial review queue—needlessly delaying final publication.

Incredibly, three final submissions (12% of the total) intended for MYCOTAXON 131(4) contained wrong illustrations, which authors did not think to check until AFTER they received their PDF files. Such major errors should have been detected well before final manuscript preparation; at the very least the corrected illustrations should have been sent to the Editor-in-Chief prior to PDF conversion. Art files are not included in the errata; rather than publish scientifically erroneous papers, a very unhappy editor decided to replace the files at deadline and to access correction fees. Be warned!

MYCOTAXON 131(4) presents 24 papers by 103 authors (representing 17 countries) and revised by 34 expert reviewers.

Within its pages are five new genera (*Abieticola* from South Korea, *Arthromoniliphora* from Brazil, *Discopycnothyrium* from Thailand, *Entalbostroma* from New Zealand, and *Podosporiopsis* from China) and 16 species new to science representing *Abieticola* and *Thelenella* from South Korea; *Alternaria* from the U.S.A., *Apiosordaria*, *Blodgettia*, *Nawawia*, *Podosporiopsis*, *Podosporium*, *Strigula*, and *Zasmidium* from China; *Arthromoniliphora* from Brazil; *Discopycnothyrium* and *Pezicula* from Thailand; *Entalbostroma* from New Zealand, and *Phaeocollybia* from Pakistan.

In addition to range extensions and/or new hosts for previously named taxa, we offer a paper dedicated to the “jubilee of a flourishing 200-year old girl, *Alternaria*, that assigns 34 spp. to *Alternaria* sect. *Infectoriae* and 3 spp. to *A.* sect. *Pseudoalternaria* based on morphological criteria. Two other papers work to clarify *Peziza*—one comparing four troublesome species using both morphological and phylogenetic criteria and another refining the determinations of 40 collections stored in the Mycological Herbarium of Tartu (Estonia). Keys are provided for species of *Apiosordaria* worldwide, *Mucor* from Brazil’s Caatinga biome, and *Graphis* from the African Palaeotropics.

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)

16 January 2017

FOUR STEPS TO SUCCESSFUL MYCOTAXON PUBLICATION IN 2017

Prospective MYCOTAXON authors should download instructions PDF, review and submission forms, and other helpful templates by clicking the ‘file download page’ link on our INSTRUCTIONS TO AUTHORS page before preparing their manuscript. Below is a summary of our ‘4-step’ publication process.

1—PEER REVIEW: Email formatted text and illustration files with a 2017 MYCOTAXON Reviewer Comments Form to 2–3 experts for peer review. Authors should (i) ask peer reviewers to return revisions and comment forms to BOTH authors and *Editor-in-Chief* <editor@mycotaxon.com> and (ii) follow reviewer suggestions **before** sending revised files to the *Nomenclature Editor* for nomenclatural review.

2—NOMENCLATURAL REVIEW: Email all corrected, **ERROR-FREE** text and illustration files to the *Nomenclature Editor* <PennycookS@LandcareResearch.co.nz> for accession and pre-submission review. Place ‘MYCOTAXON’ + first author surname on the subject line; list all peer reviewer names+Email addresses in the message. The *Nomenclature Editor* will assign accession numbers and return annotated files with a list of final corrections to the authors and *Editor-in-Chief*.

3—FINAL SUBMISSION: Consult experts, thoroughly revise and proof-read manuscripts to prepare error-free text and image files ready for immediate publication. **LABEL** the EMAIL message and **EVERY FILE** with the accession number before attaching the (i) 2017 MYCOTAXON submission form; (ii) text files for main text, tables, and legends; (iii) final art files to the *Editor-in-Chief* <editor@mycotaxon.com> with the (iv) nomenclatural identification verification for each new name. The *Editor-in-Chief* usually Emails all coauthors and expert reviewers within two weeks of final submission, but please wait at least 14 days before sending a follow-up query (without attachments).

4—FINAL EDITORIAL REVIEW & PRESS PREPARATION: The *Editor-in-Chief*, who rejects or returns for revision files not ready for publication, conducts a final grammatical and scientific review of tentatively approved manuscripts and returns text files to all expert reviewers and coauthors with the final Mycotaxon editorial review for final author approval. After sending PDF proof, bibliographic citation, and nomenclatural entries to all coauthors for final inspection, the *Editor-in-Chief* will correct **ONLY** processing or editorial errors prior to publication but will list corrections of author errors in the ERRATA of a subsequent volume for no charge. Authors are expected to arrange payment of page charges and optional open access fees with the *Business Manager* <subscriptions@mycotaxon.com> at this time.

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***Nawawia oviformis* sp. nov. from China**

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ABSTRACT—*Nawawia oviformis*, a new species collected from decaying leaves in China, is described and illustrated. It differs from previously described *Nawawia* species in having oviform conidia with 5 setulae, one of which is on the conidial apex.

KEYWORDS—freshwater fungi, hyphomycetes, taxonomy

Introduction

Nawawia was established by Marvanová (1980) based on *Clavatospora filiformis* Nawawi (Nawawi 1973); it is characterized by septate, macronematous conidiophores, terminal phialidic conidiogenous cells, and turbinate-tetrahedral to obpyramidal unicellular hyaline conidia with a filiform appendage at each blunt corner. Six *Nawawia* species have been described, four from Malaysia (Crous et al. 2009, Goh et al. 2014, Kuthubutheen et al. 1992, Nawawi 1973), one from South Africa (Hyde et al. 1996), and one from the Russian Far East (Mel'nik & Hyde 2006).

In our survey of fungi from decaying leaves collected in a stream in China, we isolated a species of *Nawawia*, which differed from the six described species by its conidial shape.

Materials & methods

Submerged dicotyledonous leaves were collected from a stream in Damuling Mountain of Gaosun County, Sichuan Province, China. Samples were preserved in zip-

[§]These authors contributed these work equally

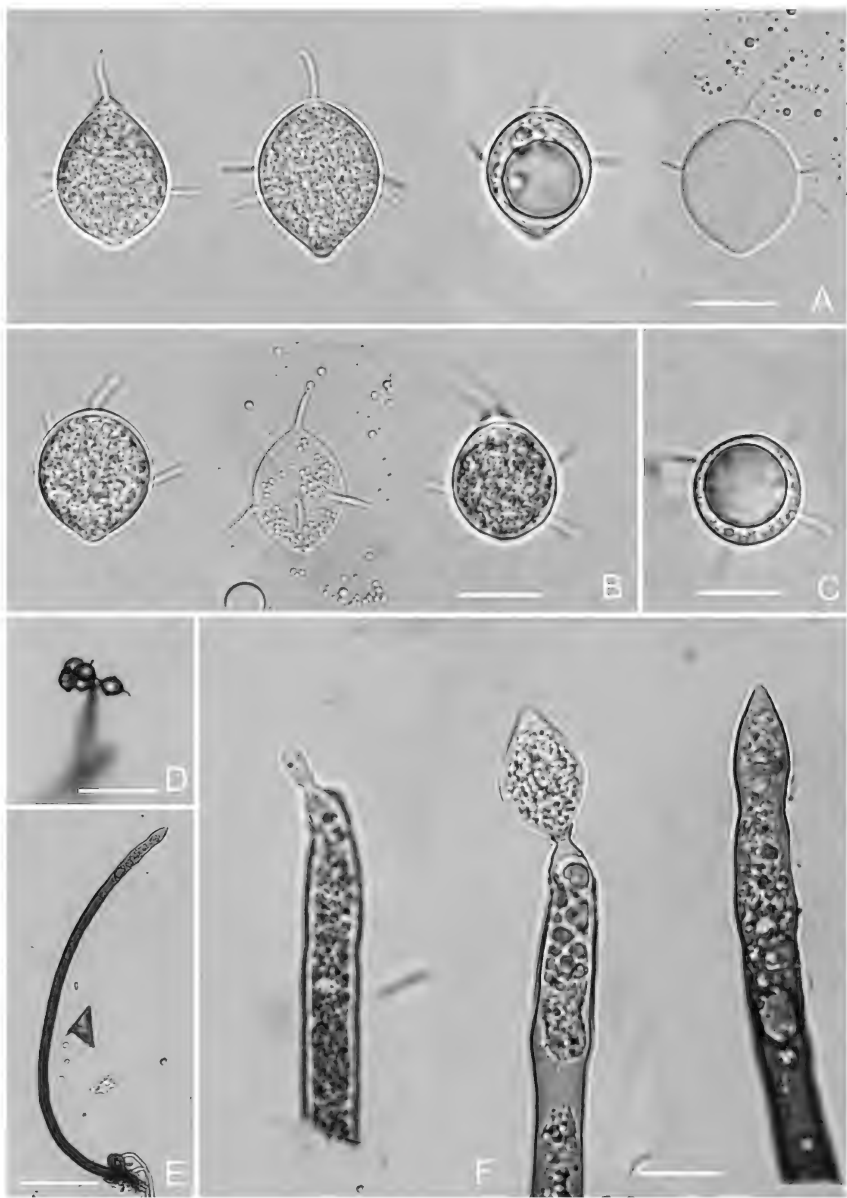


PLATE 1. *Nawawia oviformis* (holotype, YMF 1.04361). A, B. Conidia viewed from side; C. Conidium viewed from above; D. Conidia with conidiophore; E. Conidiophore; F. Conidiogenous cells. Scale bars: A–C, F = 10 µm; D, E = 50 µm.

locked plastic bags, marked, and later transported to the laboratory. By cutting several 2–4 × 2–4 cm fragments in the laboratory, the rotten leaves were spread on the surface of CMA medium (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) and incubated for about 10 days. Single conidia were isolated using sterilized needles under a BX51 microscope, and cultivated on CMA in Petri plates; morphological observations were made after incubation at 28°C for one week. A metabolically inactive frozen pure culture was deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF; formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan).

Taxonomy

Nawawia oviformis J. Peng & Z.F. Yu, sp. nov.

PLATE 1

MYCOBANK MB815592

Differs from other *Nawawia* spp. by its oviform conidia.

TYPE: PR China, Sichuan Province, Gaosun County, Damuling Mountain, 28°92'N 103°88'E, elev. 513 m, on submerged leaves of an unidentified dicotyledonous plant in a stream, July 2015, Z.F. Yu (Holotype, metabolically inactive frozen culture YMF 1.04361).

ETYMOLOGY: Latin, *oviformis* referring to the egg-shaped conidia.

COLONIES on CMA, reaching about 30 mm diameter after 10 days at 25°C. Mycelium scanty superficial, mostly immersed composed of branched, septate, hyaline hyphae. CONIDIOPHORES arise from a small stromatic cushion, cylindrical, single, unbranched, erect, robust, thick-walled, smooth, dark brown, becoming paler towards the apex, 10–13-septate, 246–323 × 4–9 µm. CONIDIOGENOUS CELLS phialidic integrated, terminal, determinate, brown, 21–60 × 3.5–9 µm. CONIDIA oviform to somewhat globose, slightly truncate at the base, unicellular, smooth, hyaline, guttulate, 8–10.5 µm diam. 13–20 × 11–15 µm, with four equatorial filiform appendages and an apical filiform appendage, all 3–10 µm long.

Discussion

Among the previously described *Nawawia* species, *N. dendroidea*, *N. filiformis*, *N. nitida*, and *N. sasae-kurilensis* have round–tetrahedral or obpyramidal conidia that are distally 3-lobed and mostly with three apical setulae, *N. quadrisetulata* has conidia that are distally 4–5-lobed and mostly with four apical setulae, and *N. malaysiana* has conidia that are distally 4–5-lobed and mostly with one basal and four apical setulae.

Acknowledgements

This work was jointly financed by National Basic Research Program of China ('973' Program: 2013CB127506) and National Natural Science Foundation Program

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Pezicula chiangraiensis sp. nov. from Thailand

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ABSTRACT—A sexual morph of a new species, *Pezicula chiangraiensis*, was collected on bark of decaying wood in Chiang Rai Province, Northern Thailand. Morphologically it is closely related to *P. cinnamomea* but differs by its ascospores having a gelatinous sheath; in culture it produces a sporodochium-like asexual morph. Phylogenetic analysis of combined ITS, LSU, and RPB2 sequence data confirmed that *P. chiangraiensis* is distinct from other *Pezicula* spp. The new species is described, illustrated, and compared with similar taxa.

KEY WORDS—*Cryptosporiopsis*, *Dermateaceae*, discomycetes, phylogeny, taxonomy

Introduction

Pezicula Tul. & C. Tul. (*Leotiomyces*, *Helotiales*, *Dermateaceae*), a widespread discomycete genus typified by *Peziza carpinea* Pers., was established by Tulasne & Tulasne (1865). Verkley (1999) accepted 26 species in *Pezicula*.

Subsequently, two new species and nine new combinations have been added to the genus (Johnston et al. 2014, Yuan & Verkley 2015, Zhong et al. 2001).

Pezicula species are characterized mainly by brightly coloured apothecia, which occur on the bark of woody plants (Ooki et al. 2003). The apothecia are frequently sessile, erumpent, and occasionally short-stalked with circular discs (Verkley 1999). The disc, initially concave and surrounded by slightly elevated margins, later becomes convex in most species (Verkley 1999). Most *Pezicula* species have 8-spored, clavate, or cylindric-clavate inoperculate asci. Ascospores vary in shape from broadly ellipsoid to elongately ellipsoid to allantoid or fusoid (Ooki et al. 2003, Verkley 1999).

Cryptosporiopsis has been reported as the asexual morph of *Pezicula* and 20 of the 26 *Pezicula* species accepted by Verkley (1999) are known to have *Cryptosporiopsis* asexual morphs. *Cryptosporiopsis* species produce sporodochial conidiomata, and most produce both macroconidia and microconidia that form on different conidiophores (Verkley 1999).

Pezicula and *Cryptosporiopsis* species are generally known as plant pathogens (Ooki et al. 2003). One *Cryptosporiopsis* species causes twig rot of cherry (Koiwa & Yamada 1996), and *C. corticola* (Edgerton) Nannf., the asexual morph of *Pezicula corticola* (C.A. Jørg.) Nannf., is reported to cause bull's-eye rot of Japanese pear. *Pezicula cinnamomea* (DC.) Sacc. causes stem canker of apple and pear (Nitta et al. 2002). *Pezicula* species are also endophytes (Kowalski & Kehr 1996). They play an important role in natural pruning that helps to produce a clean bole by decaying and shedding of dead branches (Kowalski & Kehr 1996). Species in this genus predominantly colonize woody twigs, although some occur on herbaceous plants (Gené et al. 1990, Kowalski & Bartnik 1995, Kowalski et al. 1998, Sankaran et al. 1995, Zhong et al. 2001). Most *Pezicula* species have been recorded from temperate and boreal forests of the northern hemisphere (Verkley 1999), with only a few species described from the southern hemisphere (Sankaran et al. 1995, Zhuang & Korf 1988).

Here we provide detailed morphological descriptions of sexual and asexual morphs of a new species, *Pezicula chiangraiensis*, and morphological comparisons with other closely related taxa. We also provide a multi-gene phylogenetic tree for *Pezicula* to infer the phylogenetic relationships of *P. chiangraiensis* with other *Pezicula* spp.

Materials & methods

A collection of *Pezicula* was made in Chiang Rai Province, Northern Thailand, in January 2015. Macroscopic and microscopic characters of the specimen were recorded. A Motic SMZ-168 stereomicroscope was used to observe the structure of the apothecia. Sections of apothecia were made by hand with a razor blade and mounted in water

and preserved in lacto-glycerol. A Nikon ECLIPSE 80i compound microscope was used to observe microscopic characters. Indian ink was added to detect the presence of mucilaginous sheaths. The amyloidity of asci was tested using Meltzer's reagent. Photomicrography was carried out with a Canon 450D digital camera fitted to the microscope. Measurements of apothecia, paraphyses, asci, and ascospores were made from material mounted in water. Measurements were made with the Taro soft (R) Image Frame Work v. 0.9.7 program and images used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems).

TABLE 1 *Loramyces* and *Pezicula* species used in the phylogenetic analysis.
New sequences are in bold.

SPECIES	CULTURE NO.	GENBANK ACCESSION NO.		
		ITS	LSU	RPB2
<i>L. macrosporus</i>	AFTOL-ID 913	JN033383	DQ470957	DQ470907
<i>P. acericola</i>	CBS 239.97	KF376154	KR858884	KF376214
	CBS 245.97	KF376153	KR858889	KF376213
<i>P. aurantiaca</i>	CBS 201.46	KF376150	KR858893	KF376210
<i>P. californiae</i>	CBS 124805	KR859104	KR858895	KR859332
	CBS 124819	GU973504	GU973597	–
<i>P. carpinea</i>	CBS 324.97	KF376156	KR858896	KF376160
	CBS 921.96	KF376155	KR858898	KF376159
<i>P. chiangraiensis</i>	MFLUCC 15-0170	KU310621	KU310622	KU310623
<i>P. cinnamomea</i>	CBS 239.96	KF376102	KR858915	KF376165
	CBS 625.96	KF376104	KR858943	KF376164
	CBS 240.96	KF376105	KR858916	KF376163
	CBS 626.96	KF376103	KR858944	KF376162
<i>P. corni</i>	CBS 285.39	AF141182	–	–
<i>P. corticola</i>	CBS 259.31	AF141179	KR858956	–
	CBS 260.31	KR859165	KR858957	–
<i>P. corylina</i>	CBS 249.97	KF376106	KR858960	KF376161
<i>P. diversispora</i>	CBS 185.50	KR859170	KR858962	–
	CBS 282.47	KR859171	KR858963	–
<i>P. eucrita</i>	CBS 656.96	KF376144	KR858977	KF376208
	CBS 257.97	KR859177	KR858969	–
<i>P. frangulae</i>	CBS 778.96	KF376151	KR859001	KF376212
	CBS 100244	KF376152	KR858996	KF376211
<i>P. livida</i>	CBS 262.31	AF141180	–	–
<i>P. melanigena</i>	CBS 898.97	KR859211	KR859003	–
<i>P. neosporulosa</i>	CBS 724.95	KR859231	KR859023	–
	CBS 660.95	KR859228	KR859020	–
	CBS 101.96	KR859223	KR859015	–
	CBS 949.97	KF376149	KR859025	KF376215
<i>P. pruinosa</i>	CBS 292.39	AF141188	KR859026	–
<i>P. rubi</i>	CBS 253.97	KF376100	KR859042	KF376204
	CBS 593.96	KF376101	KR859045	KF376203
<i>P. sporulosa</i>	CBS 224.96	AF141172	KR859053	KF376201
	CBS 225.96	KF376107	KR859054	KF376202
<i>P. subcarnea</i>	CBS 203.46	AF141171	KR859059	–

Observation of cultures and asexual morph

A pure culture was obtained from single spore isolation as described by Chomnunti et al. (2014). Germinating spores were transferred to malt extract agar (MEA) and incubated at 25 °C for one week. Cultural characteristics, such as mycelium colour, shape and texture, were determined. After one week, hyphal tips were cut and transferred to new media. The cultures were incubated at 25 °C under light for one week and growth rate was measured. After one week, cultures on MEA were checked for asexual structures. Conidiophores, conidiogenous cells and conidia were observed and measured by phase contrast microscopy under 400× and 1000× optical magnifications. The type specimen was deposited in the Mae Fah Luang University Herbarium, Chiang Rai, Thailand (MFLU) and an ex-type culture in Mae Fah Luang University Culture Collection (MFLUCC). A Faces of Fungi number was obtained as described in Jayasiri et al. (2015).

DNA isolation, PCR, and sequencing

The fungal isolate was grown on potato dextrose agar (PDA) for 7–21 days at 25 °C. Total genomic DNA was extracted as described by Thambugala et al. (2015). Polymerase chain reactions (PCR) were carried out using three partial gene regions: ITS4 and ITS5 (White et al. 1990) for the internal transcribed spacer (ITS); LROR and LR5 (Vilgalys & Hester 1990) for the nuclear ribosomal large subunit (LSU); and rRPB2-5F and rRPB2-7cR (Liu et al. 1999) for RNA polymerase II (RPB2). The PCR mixtures (25 µL) contained ddH₂O (9.5 µL), PCR Master Mix (TIANGEN Co., China) (12.5 µL; 2×), DNA template (1 µL), each primer (1 µL; 10 µM). Amplification conditions for all regions comprised an initial denaturation step at 94 °C for 5 min and final extension step at 72 °C for 10 minutes. ITS amplification comprised 34 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds and elongation at 72 °C for 1 minute. LSU and RPB2 amplification comprised 37 cycles of denaturation at 94 °C for 1 minute, annealing at 54 °C for 50 seconds, and elongation at 72 °C for 1 minute. The PCR products were viewed on 1% agarose electrophoresis gels stained with ethidium bromide. The PCR products were purified and sequenced at Invitrogen Biotechnology Co., Shanghai, China.

Sequence alignment and phylogenetic analysis

Newly generated sequences were subjected to a standard BLAST search of GenBank for rough identification. Eighty-nine sequences belonging to three gene regions (ITS, LSU, RPB2) from representative *Pezicula* species and the outgroup taxon *Loramycetes macrosporus* Ingold & B. Chapm. were downloaded from GenBank (TABLE 1); these sequences were published by Abeln et al. (2000), Chen et al. (2016), Spatafora et al. (2006), and Verkley (1999). The newly generated sequences were deposited in GenBank (TABLE 1). The consensus sequences for each gene were aligned using MAFFT v. 6.864b (<http://mafft.cbrc.jp/alignment/server/index.html>). The single alignment was improved manually where necessary using BioEdit (Hall 2004). The single gene alignments were concatenated into a combined dataset. Maximum likelihood phylogenetic analyses were performed in CIPRES web portal (Miller et al. 2009) using RAXML-HPG Black Box (8.2.4) tool (Stamatakis 2006). The combined gene analysis was carried out using default conditions. The best scoring tree was selected with a final likelihood value of -7794.684804. The resultant tree was viewed with FigTree v.1.4.0

(<http://tree.bio.ed.ac.uk/software/figtree/>). Maximum Likelihood bootstrap values $\geq 60\%$ are given near the nodes (FIG. 1).

Molecular phylogeny

A backbone tree for the genera *Pezicula*, *Cryptosporiopsis*, and *Neofabraea* was generated using ITS sequence data (data not shown). Two basic clades, the *Pezicula*–*Cryptosporiopsis* clade and the *Neofabraea* clade, were identified in the backbone tree, and *Pezicula Chiangraiensis* grouped with other *Pezicula* species within the *Pezicula*–*Cryptosporiopsis* clade. A single gene tree for the genus *Pezicula* was generated from ITS sequence data (data not shown). As the single gene tree shows several taxonomic disagreements, a combined ITS, LSU and RPB2 dataset comprising 34 strains of *Pezicula* species with *Loramyces macrosporus* (CBS 235.53) as outgroup was generated to determine the exact species placement of our strain (FIG. 1). The resulting tree supports our new isolate in a distinct sister clade to *Pezicula cinnamomea* with strong bootstrap support (93%).

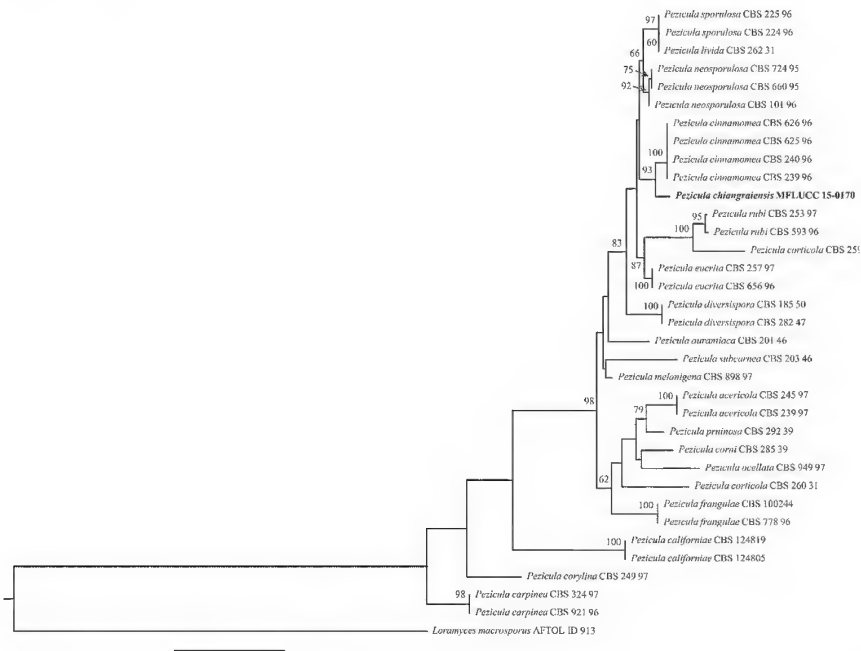


FIG. 1. The best scoring RAxML maximum likelihood phylogenetic tree based on a combined ITS, LSU, and RPB2 sequence dataset. Strain/culture numbers are given following the taxon names. The newly generated sequences are in **bold**. Bootstrap support values for maximum likelihood $>60\%$ are given near the nodes. The tree is rooted with *Loramyces macrosporus* (CBS 235.53).

Taxonomy

Pezicula chiangraiensis Ekanayaka & K.D. Hyde, sp. nov.

PLATES 1, 2

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Differs from other *Pezicula* species by possessing ascospores with a gelatinous sheath.

TYPE: Thailand, Chiang Rai Province, Kun Korn waterfall, 21 January 2015, A.H. Ekanayaka (Holotype, MFLU 15-3566; ex-type culture, MFLUCC 15-0170; GenBank KU310621, KU310622, KU310623).

ETYMOLOGY: With reference to the province where the holotype was collected.

FACESOFFUNGI: FoF 01667

SEXUAL MORPH: SAPROBIC on dead stems. APOTHECIA 800–870 × 230–280 µm ($x = 826 \times 253$ µm, $n = 10$), arising singly or in small groups, sessile, slightly erumpent from the substrate, subsphaerical, urceolate, bright yellow when fresh, brownish yellow when dried. DISC convex. RECEPTACLE yellow to light brown when fresh. ECTAL EXCIPULUM variably thick, composed of large thin-walled yellow cells in a textura angularis. MEDULLARY EXCIPULUM thin, not clearly distinguishable, composed of vertically arranged rows of yellow cells in a textura prismatica. HYMENIUM hyaline. PARAPHYSES 1.8–2.1 µm diam. ($x = 2.0$ µm, $n = 20$), numerous, filiform, obtuse, septate, branched, slightly enlarged at the apex. ASCI 90–120 × 15–25 µm ($x = 108 \times 19$ µm, $n = 30$), 8-spored, unitunicate, cylindric-clavate, short pedicellate, arising from croziers with conical and non-amyloid apices. ASCOSPORES 18–22 × 8–10 µm ($x = 20 \times 9$ µm, $n = 40$), partially biseriate, lower spores uniseriate, hyaline to grey, fusoid, gelatinous sheath present; immature spores non-septate and filled with guttules; mature spores 3-septate, aguttulate, and smooth-walled.

ASEXUAL MORPH: CONIDIOMATA sporodochium-like, produced in 7-day-old cultures, gregarious, dark brown. CONIDIOPHORES 11–12 × 5–6 ($x = 11.5 \times 5.8$ µm, $n = 20$), branched near the base, septate, smooth-walled, composed various size cells. CONIDIOGENOUS CELLS holoblastic, cylindrical, smooth, hyaline. CONIDIA 23–29 × 11–12 µm ($x = 25.2 \times 11.2$ µm, $n = 20$), cylindrical, straight, smooth with rounded apex and slightly truncate base, multi-guttulate or filled with granular cytoplasm, hyaline and aseptate when immature, pale brown and 1–3-transversely septate at maturity.

CULTURE CHARACTERISTICS: Ascospores germinating on MEA within 24 h. Colonies on MEA plates at 25–27 °C reaching 2 cm diameter within 1 week, circular, flat or effuse, with diffuse edge, medium honey-brown, grey aerial mycelium surrounded by pink mycelia, mycelium composed of 2–3 µm wide, septate, branched, smooth, hyaline hyphae, reverse dark brown to black.

NOTES—*Pezicula cinnamomea* is morphologically similar to *P. chiangraiensis* in having apothecia, paraphyses, asci, and ascospores of a similar size and



PLATE 1. *Pezicula chiangraiensis* (holotype, MFLU 15-3566), sexual morph: a. Substrate; b. Ascomata on wood; c. Ascoma; d. Cross section of ascoma; e. Vertical section of ascomal margin; f. Septate paraphyses; g-j. Short pedicellate asci; k. Germinated ascospore; l. Apex of mature ascus; m. Ascospore with gelatinous sheath; n-q. Fusoid ascospores. Scale bars: b = 500 µm; c, e = 100 µm; d = 300 µm; f, k = 50 µm; g-j = 40 µm; l, m = 20 µm; n-q = 10 µm.

shape, but differs by its pale luteous to cinnamon or red-brown apothecia, its transversely and longitudinally septate spores without gelatinous sheaths, its faster growth rate, and absence of diffusing pigment in culture (Ooki et al. 2003,



PLATE 2. *Pezicula chiangraiensis* (MFLUCC 15-0170), asexual morph in culture: a. 7-day-old MEA colony from above; b. 7-day-old MEA colony from below; c. Conidiomata; d. Conidioma; e. Septate and branched hyphae; f. Fragment of small sporodochium-like conidioma; g, h. Different stages of conidiogenesis; i–l. Conidia at different stages. Scale bars: e = 50 μm ; f, j–l = 20 μm ; g, h = 30 μm ; i = 10 μm .

Verkley 1999). *Pezicula aesculea* Kirschst. differs from *P. chiangraiensis* by its larger apothecia, asci, and ascospores; *P. amoena* Tul. & C. Tul. by its dark brown, thick-walled ascospores; *P. heterochroma* Verkley, *P. frangulae* (Pers.) Fuckel, *P. rubi* (Lib.) Niessl, *P. puberula* (E.J. Durand) Verkley, *P. crataegicola* (E.J. Durand) J.W. Groves, *P. myrtillina* (P. Karst.) P. Karst., and *P. grovesii* Wehm. by their short, stout stalked apothecia; *P. linda* Korf and

P. sepium (Desm.) Dennis by their asci with an amyloid ring; *P. tasmanica* W.Y. Zhuang & Korf by its positive reaction with Meltzer reagent after pretreating with KOH; *P. hamamelidis* J.W. Groves & Seaver by its broader apothecial base that is entirely hidden under the bark of its host substrate; and *P. melastomatis* Rehm by its smaller asci and ascospores (Verkley 1999). The asexual morphs of most *Pezicula* species produce both macro and microconidia (Verkley 1999), but microconidia were not observed in *P. chiangraiensis*.

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***Abieticola koreana* gen. et sp. nov., a griseofulvin-producing endophytic xylariaceous ascomycete from Korea**

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ABSTRACT—A new genus and species, *Abieticola koreana*, is described. This xylariaceous fungus was isolated from the inner bark of a Manchurian fir (*Abies holophylla*) in Korea. Phylogenetic analyses based on the sequences of four gene regions—ITS1-5.8S-ITS2, 28S, β -tubulin, and rpb2—were used to confirm this new genus and species.

KEY WORDS—*Ascomycota*, anamorph, multigene, Xylarioideae, *Xylariaceae*

Introduction

The *Xylariaceae* is a large family of ascomycetes comprising approximately 85 genera and at least 1,340 species that are distributed worldwide and exhibit exceptional diversity in the tropics (Whalley 1996, Velmurugan et al. 2013, Stadler et al. 2014). It has been estimated that the *Xylariaceae* contains 10,000 undescribed species (Stadler 2011, Richardson et al. 2014). Recent phylogenetic studies (Tang et al. 2009, Daranagama et al. 2015) using combined ITS, LSU, rpb2, and β -tubulin sequences, suggested that *Xylariaceae* has two major lineages, Xylarioideae and Hypoxyloideae.

To date, 11 genera of *Xylariaceae* have been shown to occur as endophytes of various plants (Pažoutová et al. 2013), including firs, palms, orchids, bromeliads, aroids, ferns, and rainforest trees (Dreyfuss & Petrini 1984, Bayman

et al. 1998; San Martín et al. 2001; Park et al. 2005). Recently, endophytes have been predominantly investigated because of their ability to produce either new or interesting secondary metabolites and for their potential use in bioenergy applications (Bayman et al. 1997, Isaka et al. 2000, Boonphong et al. 2001, Wiyakrutta et al. 2004, Park et al. 2005, Liu et al. 2007).

During screening for organisms that produce antifungal agents, an endophytic fungus designated as F0010, was isolated from the inner bark of *Abies holophylla* (Manchurian fir), a tree that is widespread in Korea (Park et al. 2005). Initially, the isolate was provisionally identified as an unknown species of *Xylaria* and was shown to produce two antifungal metabolites, dechlorogriseofulvin and griseofulvin (Park et al. 2005). However, further investigation suggested that F0010 differs from species classified in *Xylaria* based on both morphological details of the anamorph and on molecular phylogenetic analyses.

The objectives of this study were to image the ultrastructure of the conidial state of F0010 by scanning electron microscopy (SEM), for comparison of its mode of conidiogenesis with that of other *Xylariaceae*, and to investigate its phylogenetic relationship to other xylariaceous fungi based on a multigene analysis, including the nuclear ribosomal ITS1-5.8S-ITS2 and 28S subunit, *rpb2*, and β -tubulin gene sequences. As a result of this study, a new genus *Abieticola* and species *A. koreana* are proposed here to accommodate this novel xylariaceous fungus.

Materials & methods

Fungal isolation

Samples of *Abies holophylla* bark were collected from a garden in Daejeon, Korea, placed in zip-lock bags, stored in a refrigerator, and then used for the isolation of endophytic fungi within 72 h after sampling. Samples were cleaned under running tap water and then air-dried. The samples were surface sterilised by immersion in 70% ethanol for 1 min and a 3.5% sodium hypochlorite solution for 5 min. Next, the surface-sterilized samples were washed in sterile water three times to remove the surface sterilization agents (Wiyakrutta et al. 2004). The samples were then cut into small pieces and placed on cornmeal malt extract agar (17.0 g of cornmeal, 20.0 g of malt extract, 2.0 g of yeast extract, and 20 g agar in 1.0 L of distilled water) supplemented with chloramphenicol (50 mg/mL) (Sigma, St. Louis, MO, USA), and then incubated at 25°C for up to 3 weeks. Individual fungal strains were transferred to potato dextrose agar (PDA, Bacto Potato Dextrose Dehydrated, Becton Dickinson, USA) and incubated at 25°C for at least 2 weeks. After checking for purity, each fungal culture was transferred to a fresh agar plate. Stock cultures of test species were grown on MEA at 25°C and used as inocula. The basal medium consisted of 2% malt extract agar (MEA, Difco) at pH 5.5. The water activity (a_w) of the basal MEA medium was 0.999, which was modified to 0.95, 0.98, and 0.995 by addition of KCl and glucose. The a_w of media was measured using

a Novasina ICII (Novasina AG, Zurich, Switzerland). The growth of the fungus was determined by transferring mycelial plugs (5 mm diameter) from the growing margin of stock cultures in Petri dishes (90 × 15 mm). After inoculation, plates with the same a_v were sealed in polyethylene bags and incubated at 20, 25, and 30°C in darkness for up to 10 days. Mycelial growth was recorded periodically by measuring the diameter of the colony in two directions at right angles to each other.

Ten strains, F0010-1 to F0010-10, were derived from isolate F0010. Dried MEA cultures were conserved in Chonnam National University Fungal Collection, Gwangju, Korea (CNUFC), and live cultures were conserved in CNUFC and in the Korean Collection for Type Cultures, Daejeon, Korea (KCTC).

Ultrastructure of isolate F0010

The micromorphological features of strain F0010-1 were investigated by light microscopy (LM) with a Nikon Labophot 2 Microscope (Nikon, Tokyo, Japan) with differential interference contrast optics. For scanning electron microscopy (SEM), samples were fixed in 2.5% paraformaldehyde-glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h, post-fixed with 1% OsO₄ in the same buffer for 1 h, dehydrated in graded ethanol, substituted by isoamyl acetate and then critical-point dried in CO₂. Finally, the sample was sputter-coated with gold (SC502; Polaron, West Sussex, UK) and observed with a SEM515 scanning electron microscope (Phillips, Eindhoven, the Netherlands) and an S-3500N low vacuum scanning microscope (Hitachi Science Systems Ltd., Tokyo, Japan).

DNA extraction and PCR amplification

Strains F0010-1 and F0010-2 were grown on PDA, covered with cellophane at 25°C for 4–5 days. Genomic DNA was extracted using the HiGene™ Genomic DNA Prep Kit for fungi (Biofact Co., Daejeon, Korea). The target regions, ribosomal ITS1–5.8S–ITS2, 28S subunit, β -tubulin, and rpb2 (RNA polymerase II subunit 2) were amplified by PCR (TABLE 1) in 20 μ L reaction mixtures containing 2 μ L of genomic DNA, 1.5 μ L of each primer (5 pM), 14 μ L of demineralised sterile water, and 1 μ L of PCR premix

TABLE 1. Primers and annealing conditions used in the molecular analysis.

LOCUS	PRIMER	SEQUENCE (5'–3')	ANNEALING TEMP (°C)	REFERENCE
28S rDNA	LR5F	GCTATCCTGAGGGAAC	55 (30 sec)	Vilgalys & Hester 1990
	LR0R	ACCCGCTGAACTTAAGC		
β -tubulin	Bt2a	GGTAACCAAATCGGTGCTGCTTC	58 (1 min)	Sang et al. 2010
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		
ITS rDNA	ITS1	TCCGTAGGTGAACCTGCGG	55 (30 sec)	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC		
rpb2	RPB2-5F_Eur	GAYGAYCGKGAYCAYTTC	50–72 (1 min), ramping 0.3°C per sec	Hibbett 2006; Houbraken et al. 2011
	RPB2-7CR_Eur	CCCATRGCTGYTTTRCCCAT		

(Bioneer, Daejeon, Korea) using the following fungal-specific primer sets: ITS1 and ITS4 for ITS1-5.8S-ITS2, LROR and LR5F for 28S (White et al. 1990), Bt2a and Bt2b for β -tubulin (Sang et al. 2010), and rPB2-5F-Eur and rPB2-7cr-Eur for rPB2 (Hibbett 2006, Houbraken et al. 2011).

DNA sequencing and phylogenetic analyses

Amplified PCR products of strains F0010-1 and F0010-2 were electrophoresed on a 0.75% agarose gel. The amplicons were purified with the AccuPrep PCR Purification Kit (Bioneer). The purified PCR products were sequenced using an ABI 3700 automated DNA sequencer (Applied Biosystems Inc., USA). The four gene sequences generated were initially aligned with other sequences from GenBank using Clustal X v. 2.0 (Larkin et al. 2007) with a gap opening penalty of 10.0 and a gap extension penalty of 0.05. BioEdit v. 7.2.5 (Hall 1999) was used to manually exclude ambiguous and uninformative variable sites. The alignment was manually refined using MEGA v. 6.0 (Tamura et al. 2013). The ITS, 28S, β -tubulin, and rpb2 gene sequences of F0010-1 and F0010-2 were deposited in GenBank (see TABLE 2).

TABLE 2. Sequences used in the phylogenetic multigene analysis.

TAXON	SAMPLE	GENBANK ACCESSION NO.			
		ITS	LSU	rpb2	β -tubulin
<i>Abieticola koreana</i> (T)	EML-F0010-1	JN977612	JQ014618	KP792128	KP792126
<i>A. koreana</i>	EML-F0010-2	KP835547	KP835546	KP792129	KP792127
<i>Annulohypoxylon moriforme</i> var. <i>microdiscum</i>	CBS123834	DQ631935	DQ840061	DQ631960	DQ840095
<i>A. stygium</i>	MFLUCC 12-0826	KJ940870	KJ940869	KJ940868	KJ940867
<i>Anthostomella brabeji</i>	CBS 110128	EU552098	EU552098	—	—
<i>A. proteae</i>	CBS 110127	EU552101	EU552101	—	—
<i>Astrocystis concavispора</i>	MFLUCC 14-0174	KP297404	KP340545	KP340532	KP406615
<i>A. mirabilis</i>	HAST 94070803	GU322448	—	GQ844835	GQ495941
<i>A. sublimbata</i>	HAST 89032207	GU322447	—	GQ844834	GQ495940
<i>Collodiscula japonica</i>	CBS 124266	JF440974	—	—	JF440974
<i>Daldinia concentrica</i>	CBS 113277	AY616683	—	KC977274	—
<i>D. decipiens</i>	CBS 122879	JX658441	—	—	AY951694
<i>D. loculata</i>	BCRC 34117	EF026145	—	AY951698	—
<i>Discoxylaria myrmecophila</i>	JDR 169	GU322433	—	GQ844819	GQ487710
<i>Entoleuca mammata</i>	JDR 100	GU300072	—	GQ844782	GQ470230
<i>Euepixylon sphaeriosomum</i>	JDR 261	GU292821	—	GQ844774	GQ470224
<i>Hypoxylon fendleri</i>	MFLUCC 12-0816	KM017563	KM017565	KM017566	KM017564

<i>H. fragiforme</i>	MUCL 51264	KM186294	KM186295	KM186296	KM186293
<i>H. lenormandii</i>	MFLUCC 13-0120	KM039135	KM039136	KM039137	KM039138
<i>H. monticulosum</i>	MFLUCC 12-0818	KM052716	KM052717	KM052719	KM052718
<i>Kretzschmaria guyanensis</i>	HAST 89062903	GU300079	—	GQ844792	GQ478214
<i>Lunatiannulus irregularis</i>	MFLUCC 14-0014	KP297398	KP340540	KP340526	KP406609
<i>Nemania diffusa</i>	FR AT-113	DQ658238	DQ840073	DG631947	DQ840088
<i>N. serpens</i>	FR AT-114	DQ631942	DQ840075	DQ631948	DQ840086
<i>Podosordaria mexicana</i>	WSP 176	GU324762	—	GQ853039	GQ844840
<i>P. muli</i>	WSP 167	GU324761	—	GQ853038	GQ844839
<i>Poronia pileiformis</i>	WSP 88113001	GU324760	—	GQ853037	GQ502720
<i>Rhopalostroma lekae</i>	MFLUCC 13-0123	KJ472428	KJ472427	KJ472429	—
<i>Rosellinia lamprostoma</i>	HAST 89112602	EF026118	—	GQ844778	EF025604
<i>R. merrillii</i>	HAST 89112601	GU300071	—	GQ844781	GQ470229
<i>R. necatrix</i>	HAST 89062904	EF026117	—	GQ844779	EF025603
<i>R. sancta-cruciana</i>	HAST 89062903	GU292824	—	GQ844777	GQ470227
<i>Rostrophoxylon terebratum</i>	CBS 119137	DQ631943	DQ840069	DQ631954	DQ840097
<i>Ruwenzoria pseudoannulata</i>	MUCL 51394	GU053568	—	—	—
<i>Stilbohoxylon quisquiliarum</i>	YMJ 89091608	EF026120	—	GQ853021	EF025606
<i>Thamnomycetes camerunensis</i>	MUCL 51396	FN428828	—	—	—
<i>Xylaria adscendens</i>	JDR 865	GU322432	—	GQ844818	GQ487709
<i>X. bambusicola</i>	WSP 205	EF026123	—	GQ844802	AY951762
<i>X. hypoxylon</i>	CBS 122620	AM993141	KM186301	KM186302	KM186300

CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, BCRC Bioresource Collection and Research Centre, Taiwan, HAST Herbarium, Research Centre for Biodiversity, Academia Sinica, Taipei, JDR Herbarium of Jack D. Rogers, MFLCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, MUCL Mycotheque de l'Université Catholique de Louvain, Germany, YMJ Herbarium of Yu Ming Ju, WSP Washington State University, USA, EML Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, South Korea; T ex-holotype strain.

Maximum-likelihood (ML) (Felsenstein 1985) algorithms were applied using the general time reversible (GTR) model under assumption of the gamma distribution to accommodate substitution rates at the remaining sites and the starting tree was obtained via neighbor-joining with nearest neighbors. For the Maximum Parsimony analysis (MP), heuristic search method of tree bisection-reconnection (TBR) branch swapping was used. A neighbor-joining (NJ) analysis was calculated from the Kimura two-parameter model (Kimura 1980) with uniform rates as rate among sites (Saitou & Nei 1987). Bootstrap analyses were performed with 1000 resampled datasets using MEGA 6.05 (Tamura et al. 2013).

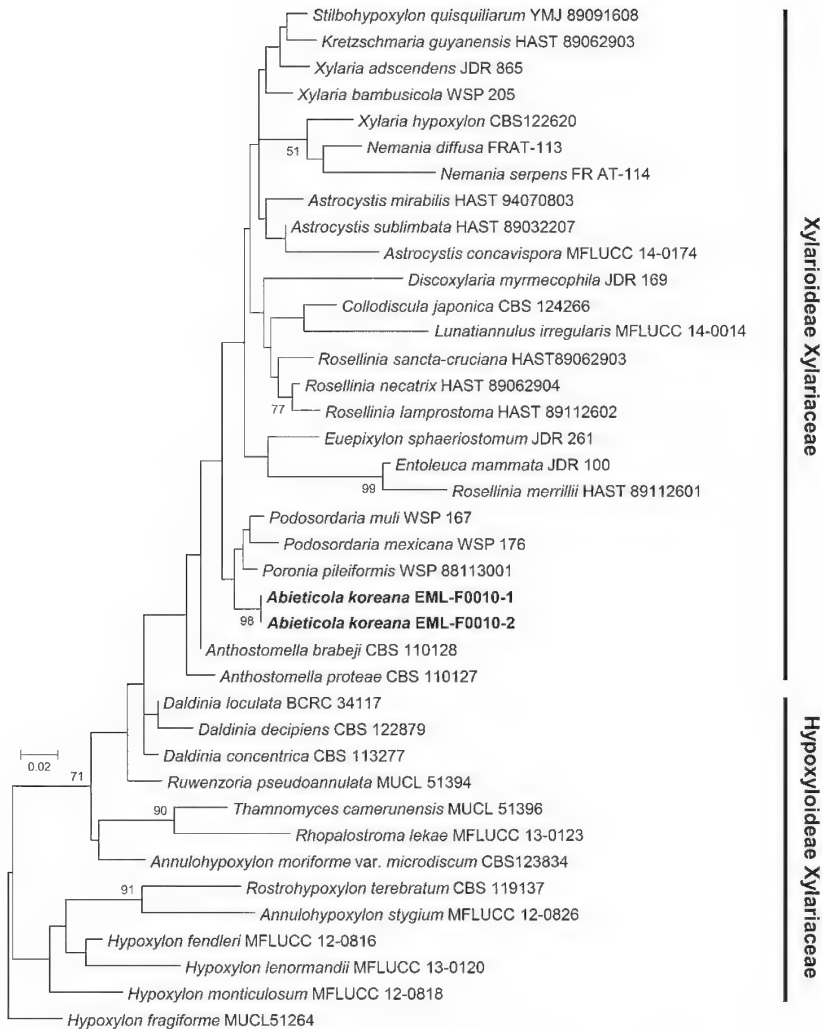


FIG. 1. Phylogenetic position of *Abieticola koreana* EML-F0010-1 and EML-F0010-2 and relationships among *Xylariaceae* based on multigene analysis (ITS, 28S, rpb2, and β -tubulin). *Hypoxyton fragiforme* was used as outgroup. The scale bar represents one substitution per 100 nucleotides. The trees were constructed based on Maximum Likelihood (ML). Phylogenetic classification by Daranagama et al. (2015).

Results

Phylogenetic analysis

F0010-1 and F0010-2 clustered within a clade including *Podosordaria* and *Poronia* species, but formed a separate monophyletic group in maximum-

likelihood (ML) trees. BLASTn search revealed that the ITS sequence of F0010-1 strain (JN977612) was 96.5% identical with *Pod. mexicana* WSP 176 (GU324762; 409 of 424 bp), 97.2% identical with *Pod. muli* WSP 167 (GU324761; 412 of 424 bp), and 96.5% identical with *Por. pileiformis* WSP 88113001 (GU324760; 409 of 424 bp). However, the sequences of F0010-1 were <95% similar to those of several isolates known to be species of *Xylaria* (FJ612908; EU716506; AY315407; EU716509). In addition, the BLASTn search results for the 28S rDNA sequence of strain F0010-1 (JQ014618) was 98.1% homologous with *Xylariaceae* sp. 1175 (FJ425703; 527 of 537 bp), 97.8% homologous with *Xylaria* sp. DIS99a (DQ327620; 715 of 731 bp), and 97.6% homologous with *Xylaria* sp. DIS255j (DQ327627; 721 of 739 bp).

To further elucidate the phylogenetic status of isolate F0010, sequence data for additional genes, *rpb2*, and β -tubulin were analysed. The BLAST results for the *rpb2* and β -tubulin gene sequences indicated that the fungus is somewhat closely related to genera such as *Podosordaria* and *Poronia*, but showing identity values of only 76.6–81.0% with *Pod. mexicana* WSP 176, 83.0–83.9% with *Pod. muli* WSP 167, and 73.2–82.2% with *Por. pileiformis* WSP 88113001. Based on the sequence data and the structure of the conidial anamorph, isolate F0010 appeared to constitute a new genus and species, forming a distinct lineage within the Xylarioideae clade, sister to the *Podosordaria*/*Poronia* lineage (FIG. 1).

Taxonomy

Abieticola Hyang B. Lee, gen. nov.

MYCOBANK MB 811702

Differs from *Poronia* by its slightly curved conidia, and its shorter conidiogenous cells.

TYPE SPECIES: *Abieticola koreana* Hyang B. Lee

ETYMOLOGY: *Abieticola*, meaning “*Abies*-inhabiting”, referring to the host genus.

A coremioid, synnematus conidial anamorph produced on artificial media is characterized by small clubs with white heads consisting of coiled conidiogenous hyphae and synnemata that resemble those of *Poronia* species but differ in bearing slightly curved conidia from shorter conidiogenous cells, some of which bear three conidia.

Abieticola koreana Hyang B. Lee, sp. nov.

FIGS 2, 3

MYCOBANK MB 811703

Differs from *Poronia* spp. by its slightly curved conidia, and its shorter conidiogenous cells that sometimes bear 3 conidia.

TYPE: Republic of Korea, Daejeon, Yuseong-gu, endophytic in inner bark of *Abies holophylla* Maxim. (*Pinaceae*), August 2006 (CNUFC EML-F0010-1 [preserved as

metabolically inactive frozen culture]; ex-type cultures, KCTC 10629BP, CNUFC EML-F0010-1; GenBank JN977612, JQ014618, KP792128, KP792126).

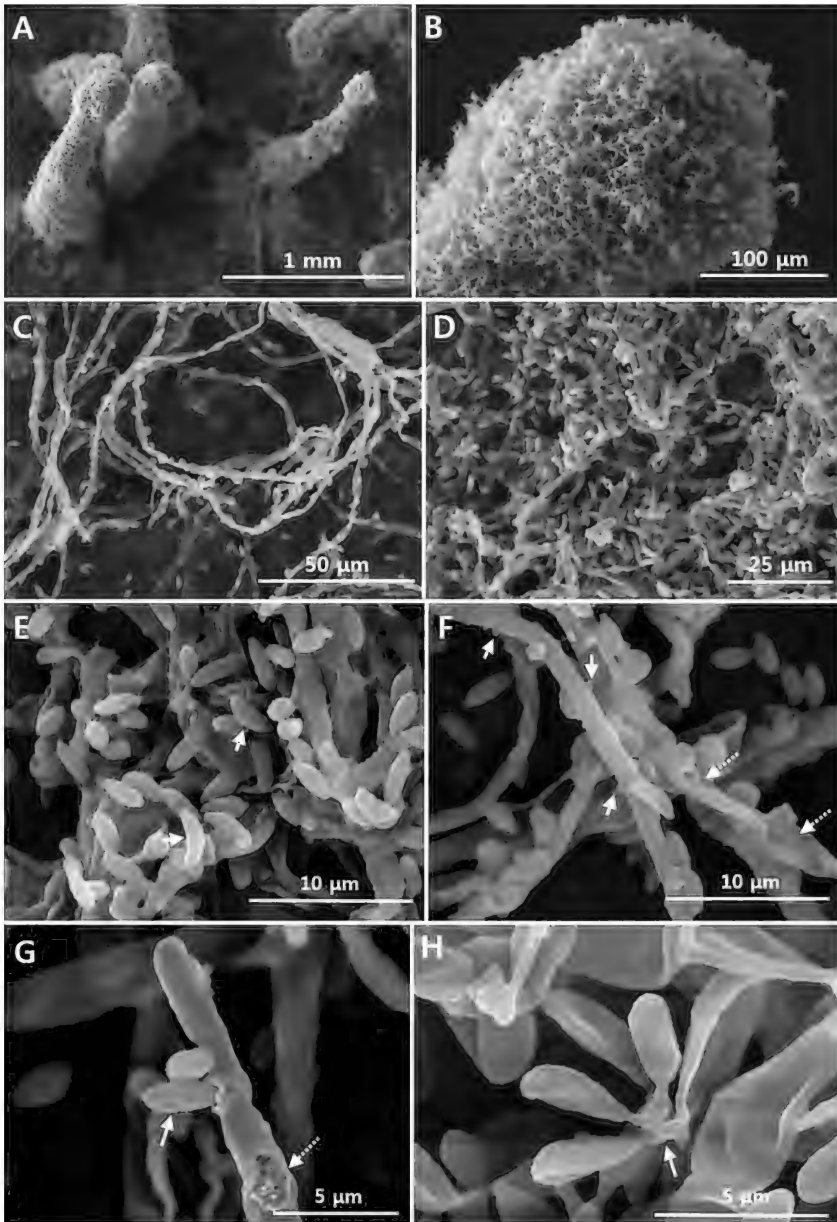
ETYMOLOGY: *koreana*, referring to the country of isolation, Korea.

STROMATA frequently developed on PDA medium, resembling small clubs with white heads, ≤ 20.5 mm tall and with blackish brown exudates oozing out along the lower stalk. Substrate mycelia forming in abundance, but aerial mycelia poorly produced. SYNNEMATA generally erect but curving in age, $8.0\text{--}20.5$ (av. 13.15 ± 1.4) $\times$ $0.9\text{--}1.4$ mm, with knotted hyphae of variable thickness (mostly $1\text{--}1.8$ μm), pigmented brown to yellow in age; functioning as conidiophores. CONIDIA 1 or 2 (sometimes 3) in number, produced laterally on mycelia and detaching with distinct scars, $2.5\text{--}4.0 \times 1.0\text{--}1.5$ (av. 3.0×1.5) μm on PDA,



FIG. 2 (above). *Abieticola koreana* (F0010-1): macromorphological culture characteristics. A, B: PDA containing 5% wild dog dung culture at 27°C for 1 month (A) and 2 months (B – plate refrigerated for 2 days and then moved to RT to induce fruiting); C, D: PDA culture at 18°C for 1 month (C) and 50 days (D); E: MEA culture with abundant mycelia and aerial club-like synnemata with long whitish heads and yellowish bases; F: culture on 5% dung medium at 27°C for 50 days; G: culture on PDA containing 5% dung at 27°C for 50 days; H: culture on MEA medium at 27°C for 1 month; I and J: culture on PDA containing 5% dung at 27°C for 50 days; K: old fruit bodies producing whitish powdery conidia and hairy mycelia (yellow arrow) in age on PDA; L: culture on PDA containing 5% dung at 27°C for 2 months (plate refrigerated for 2 days and then moved to RT to induce fruiting).

FIG. 3 (right). *Abieticola koreana* (F0010-1): micromorphological culture characteristics. A: fruit bodies of the vase-shaped structure on PDA; B: magnified picture of the round head shown in A; C: slender, long, tangled thread-like mycelia with conidia; D: magnified picture of numerous conidia



on mycelia shown in C, E: magnified pictures of anamorphic conidia in different forms including elliptical, or oblong to banana-like (solid arrow) shown in C and D, F: conidia and normal mycelia with small knots (solid arrow) and flat mycelia (dotted arrow), G: conidia (solid arrow) and scar (dotted arrow) formed on mycelia, H: conidiation in cluster (solid arrow) on mycelia.

hyaline, bases brown to yellow, tips white to grey; morphologically variable, *Poronia*-like, ellipsoid or fusoid to ovoid, often slightly curved. No teleomorph found on artificial media. COLONIES on PDA 25 mm diam. after two weeks at 25°C, yellow to brown in age.

ADDITIONAL STRAINS: REPUBLIC OF KOREA, DAEJEON, Yuseong-gu, endophytic in inner bark of *Abies holophylla*, August 2006 (CNUFC EML-F0010-2 to F0010-10).

DISTRIBUTION: Known only from the type locality in South Korea.

Growth

Colonies attained an average diameter of 25 mm on MEA medium after two weeks incubation at 25°C (the optimum temperature for growth). The medium became pigmented yellow to brown with age. Mycelial growth rates were determined under different water activity conditions (0.90–0.999 a_w ; FIG. 4. Growth of strain F0010-1 was maximal at 0.999 a_w /25°C, with a similar growth rate at 0.98 a_w , but with almost no mycelial growth at 0.90 a_w , regardless of temperature. At 0.95 a_w , the strain showed moderate growth on medium amended with glucose, but no growth on medium amended with KCl solution. The strain showed almost no mycelial growth at lowered water availability level (0.90 a_w) regardless of temperature.

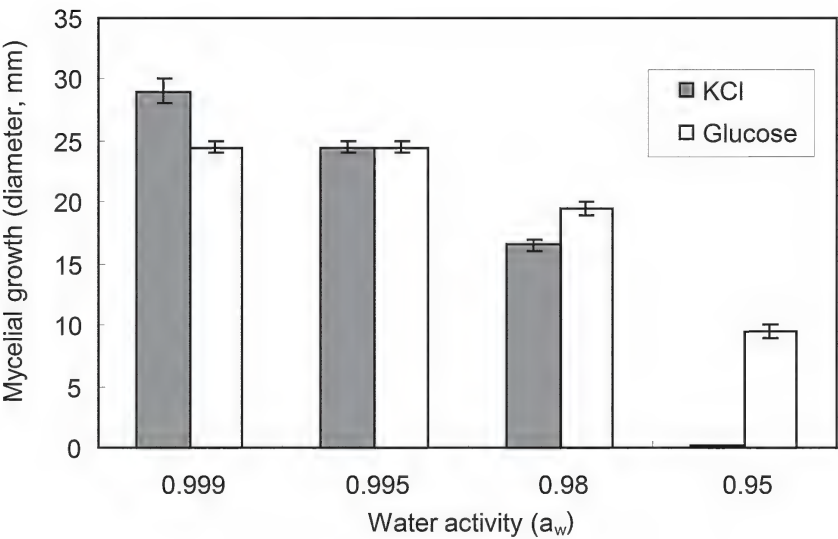


FIG. 4. *Abieticola koreana* (F0010-1): effect of water activity (a_w) on mycelial growth on PDA. The water activity levels were adjusted using two different solutes, KCl (left) and glucose (right).

Discussion

Several attempts were made to induce a teleomorph of *Abieticola koreana* on a variety of artificial media (including dung in some media because some xylariaceous fungi are coprophilous), but our attempts were unsuccessful. The conidiomata that formed in culture on artificial media were tested for germination in solution, but no spores germinated within 3 days.

Anamorphs of *Xylariaceae* differ in their modes of conidiogenesis and the complexity of the conidiophores, and these characters have previously been employed to define different form-genera (Hsieh et al. 2005). No teleomorphic structures were observed here on various artificial media including dung-containing media.

Although many related genera produce anamorphs on developing stromata, few detailed ultrastructural studies of these anamorphs have been published. The anamorphs of genera such as *Calceomyces*, *Daldinia*, *Hypoxylon*, *Rhopalostroma*, *Thamnomycetes*, and *Thuemenella* ('Hypoxylidae') have *Nodulisporium*-like anamorphs (Stadler et al. 2013); and *Xylocoremium flabelliforme* has been reported as the anamorph of *Xylaria cubensis* (Rogers 1984, Hsieh et al. 2005). The ultrastructure of the stromata and conidia and mode of conidiogenesis in *A. koreana* could be used to distinguish it from similar xylariaceous anamorphs. The mode of conidiogenesis on the surface of the synnemata of *A. koreana* was examined by SEM and found similar to other *Xylariaceae* (including those in *Podosordaria*, *Poronia*, and *Xylaria*), but the morphology of the synnemata, conidiomata, and conidia differed from those of the related genera.

Conidia formed by *A. koreana* resembled those of *Pod. leporina*, but differed slightly in that they were produced by geniculate conidiogenous cells; the elongated tips of the synnemata resembled those of *Por. ingii* in being slightly shorter but differed in the ellipsoid and subglobose conidia (Koehn & Cole 1975, Ribes & Negrin 2011). According to Rogers et al. (1998), *Podosordaria* has *Geniculosporium*-like anamorphs, while *Poronia* has *Lindquistia*-like anamorphs. *Podosordaria*, with a typically stalked stroma with a subglobose head, differs from *Poronia*, with a flattened disc (Krug & Cain 1974).

Koehn & Cole (1975) compared the ultrastructure of *Pod. leporina* and *Por. oedipus* suggesting that *Pod. leporina* should be returned to *Poronia*. The morphology and molecular phylogenetic position of *A. koreana* also differed from these two genera.

Podosordaria and *Poronia* are closely related genera containing species that have been considered close relatives of *Xylaria* but differ by their capitate stromata and coprophilous nature (Deepna & Manimohan 2012). Neighbor-joining (NJ) and maximum parsimony (MP) analyses of four DNA loci (ITS,

28S, β -tubulin, rpb2) performed in this study produced tree patterns almost identical to ML (not shown). Three ITS-based phylogenetic analyses of *Xylaria* and related genera proved to be practical for taxonomic determination of intra-specific relationships in highly morphologically variable fungi like the *Xylariaceae* (Spatafora & Blackwell 1993). Nevertheless, studies based on the ITS marker encountered major issues when attempting to determine phylogenetic relationships with other *Xylaria* species because of its highly variable nature (Hsieh et al. 2010). In addition, the number of *Xylaria*-related sequences in GenBank is limited.

Nevertheless, it is very important to note the phylogenetic status and relatedness of *Abieticola* among xylariaceous fungi because until now no intermediate group related to *Poronia* and *Podosordaria* has been reported. Results revealed that *Abieticola* is a clade within the intermediate group on phylogenetic trees such as ML, NJ, and MP.

Most *Xylaria* species ('Xylarioideae') and some segregates like *Sarcocylon* have elongate stromata; interestingly, *Daldinia asphalatum* ('Hypoxyloideae') also produce elongate stromata. The sporulating structures and attachment patterns of *A. koreana* resembled those of *Poronia* spp. (*Lindquistia*-like) and *Xylaria* spp. (*Geniculosporium*-like), but differed from them in having broader conidiogenous cells and slightly curved conidia.

The presence or absence of specific chemicals can, in many cases, be linked with taxonomic position, which may prove invaluable in determining associations between species, species groups, and genera (Whalley & Edwards 1995). The placement of the F0010-1 and F0010-2 strains in the Xylarioideae clade constructed by Daranagama et al. (2015) suggests that other members of the clade should be investigated for ability to produce griseofulvin.

Endophytic fungi are organisms that live inside plant tissue for at least part of their life cycle without causing any disease symptoms (Petrini 1991, Schulz & Boyle 2005). It is interesting that *Xylaria* species isolated from eastern white pine (*Pinus strobus*) needles and lowbush blueberry (*Vaccinium angustifolium*) stems has been reported to produce griseofulvin (Richardson et al. 2014). Liu et al. (2007) reported that an endophytic *Xylaria* sp. isolated from *Ginkgo biloba* had broad antimicrobial activity. Quang & Bach (2008) also reported that ergosta-4,6,8(14),22-tetraen-3-one produced by a *Xylaria* species from Vietnam can inhibit nitric oxide production. Several metabolites, including linoleic acid, linoleic acid methyl ester, and 4-hydroxyscytalone, were isolated from a methanolic extract of another *Xylaria* species (Jang et al. 2009). A xylariaceous coprophilous fungus, *Pod. tulasnei*, was shown to produce tulasnein, a new metabolite with strong antimicrobial activity and weak cytotoxic and phytotoxic activities (Ridderbusch et al. 2004). *Poronia punctata* has been

shown to produce a range of biologically active sesquiterpenes (Poyser et al. 1985, Anderson et al. 1988). Stadler et al. (2014) noted that fungal secondary metabolite profiles can have taxonomic value beyond the species rank and even coincide with phylogenetic data. Kuhnert et al. (2014) indicated that the biosynthetic pathways of diverse compounds derived from fungi have evolved independently during the evolution of fungi. More studies on the molecular phylogeny and ultrastructure of diverse groups belonging to the Xylarioideae clade, which are related to the new genus and species, are needed.

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***Entalbostroma erumpens* gen. et sp. nov. (Xylariaceae) from *Phormium* in New Zealand**

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ABSTRACT—A xylariaceous fungus on the dead leaves of *Phormium tenax* and *P. cookianum* is described as a new genus and species, *Entalbostroma erumpens*. Phylogenetically *E. erumpens* is close to *Xylaria amphithele*. The sexual state resembles *Kretzschmariella* in general morphology, but the two genera are phylogenetically distant. The asexual state can be used to distinguish the genera morphologically, *Entalbostroma* having an anamorph with typical xylariaceous conidia, while *Kretzschmariella* has *Mirandina*-like conidia, unknown in any other xylariaceous taxon.

KEY WORDS— phylogeny, *Xylariales*, *Xylarioideae*

Introduction

The generic taxonomy of the *Xylariaceae* remains uncertain. Phylogenetic studies have shown that few of the long recognised, morphologically based genera form well resolved, monophyletic clades, most being polyphyletic, paraphyletic, or with poorly resolved relationships in DNA based phylogenetic studies (Hsieh et al. 2010, Pažoutová et al. 2010, U'Ren et al. 2016). As discussed by U'Ren et al. (2016), resolution of phylogenetically robust genera within the *Xylariaceae* requires not only additional genes but also more complete taxon sampling.

In this paper we fill a gap in the taxon sampling of these fungi by describing a morphologically unusual xylariaceous fungus from leaves of the monocotyledonous *Phormium* spp. (*Asparagales*, *Hemerocallidaceae*) as a new genus and species. Morphologically the sexual state is similar to

Kretzschmariella (Ju & Rogers 1994); however newly obtained DNA sequences for *Kretzschmariella culmorum* show the two taxa to be phylogenetically distant.

Materials & methods

Specimens were collected during general surveys of fungi associated with the native plants of New Zealand.

Ascospore masses were removed aseptically from perithecia in the holotype specimen PDD 107494 and spotted onto SMEA medium (Kenerley & Rogers 1976). Germinated ascospores were transferred to 2% oatmeal agar (OA, Difco), Leonian's medium (Booth 1971), and half strength potato dextrose agar (PDA, Difco). Asci and ascospores from dried specimens and conidia from pure cultures were examined in water or 5% KOH with Melzer's reagent.

For DNA sequencing, mycelium was harvested from the cultures, dried, then ground in 400 µL lysis buffer (Qiagen, USA) with a plastic pestle followed by incubation for 2 hr at 55°C. Then 220 µL lysed solution was loaded into the QIAxtractor Robot (Qiagen, USA) and DNA extraction performed with a Qiagen DX reagent pack and tissue extraction protocol. DNA sequences for ITS, β -tubulin, and RPB2 were generated using the amplification primers from Hsieh et al. (2010). In addition, DNA was extracted from the hymenium of a fruit body from PDD 107494, prior to the specimen being dried for storage, and an ITS sequence generated.

The DNA sequences were concatenated and a phylogenetic analysis performed using our newly generated sequences together with selected taxa from U'Ren et al. (2016) and previously unpublished sequences from a culture grown from a collection of *Kretzschmariella culmorum* (Guadeloupe, on culm of bamboo, coll. J. Vivant, 1 Nov 1992, sent by H.W. Ravenel (#2505) to WSP; culture stored at The Institute of Plant and Microbial Biology, Academia Sinica). An initial analysis showed that *Entalbostroma erumpens* was within the *Xylaria* 'HY' clade of U'Ren et al. (2016). The taxa selected were those that represented the diversity across that clade, together with representative taxa from the other clades recognised within the Xylarioideae by U'Ren et al. *Poronia pileiformis* and *Podosordaria mexicana* were used as the outgroup. The sequences were aligned using MAFFT as implemented in Geneious R9 (Kearse et al. 2012). A Bayesian phylogenetic tree was generated using MrBayes 3.2 (Ronquist et al. 2012) with gaps treated as missing data, applying the GTR+I+G model for all three genes, the same model selected for all genes using the Akaike information criterion method in MrModeltest 2.3 (Nylander 2004). The data set was run with four chains for 10 million generations, trees sampled every 1000 generations with a burn-in of 25%. Bayesian posterior probabilities were obtained from 50% majority rule consensus trees.

Taxonomy

Entalbostroma J.D. Rogers & P.R. Johnst., **gen. nov.**

MYCOBANK MB817225

Differs from *Kretzschmariella* in having typical xylariaceous conidia.

TYPE: *Entalbostroma erumpens* J.D. Rogers & P.R. Johnst.

ETYMOLOGY: For the white entostroma.



FIGURE 1 *Entalbostroma erumpens* (A–C, holotype, PDD 107494; D, E, I–K, PDD 107550; F–H, ICMP 21152). A, B, fruiting bodies erumpent from leaf, variable in shape. C, fruiting body in section showing white internal tissue and continuous narrow black outer layer. D, erumpent, partly exposed fruiting body showing barely differentiated ostioles. E, fruiting body from D with upper part removed to show internal structure; F, detail of surface of culture on PDA, darkened structures, and powdery layer of conidia in places. G, conidia from PDA culture. H, conidiogenous cells from PDA culture. I, ascus apex in KOH and Melzer's reagent. J, ascospores with gelatinous sheath and small apical gelatinous cap. K, ascospores with spore-length germ slit. Scale bars: A–F = 1 mm; G–K = 10 µm.

Stromata pulvinate to applanopulvinate, orbicular to elliptical to irregular, solitary to gregarious, erumpent becoming superficial; surface blackish, interior perithecium-bearing tissue white. Perithecia more or less spherical;

ostioles ill defined. Ascus apical ring inverted hat-shaped, bluing in Melzer's iodine reagent. Ascospores dark brown, with germ slit on flattened side, a hyaline gelatinous sheath. Paraphysate.

In culture, conidiophores forming a palisade-like layer of short, cylindric cells with conidia produced holoblastically on several scars near apex. Conidia hyaline, smooth, ellipsoid with narrowed flattened bases.

***Entalbostroma erumpens* J.D. Rogers & P.R. Johnst., sp. nov.**

FIG. 1

MYCOBANK MB817226

Differs from *Xylaria amphithele* by its initially immersed, then erumpent, non-stipitate ascumata, its non-erumpent perithecia, and its ascospores being surrounded by a gelatinous sheath.

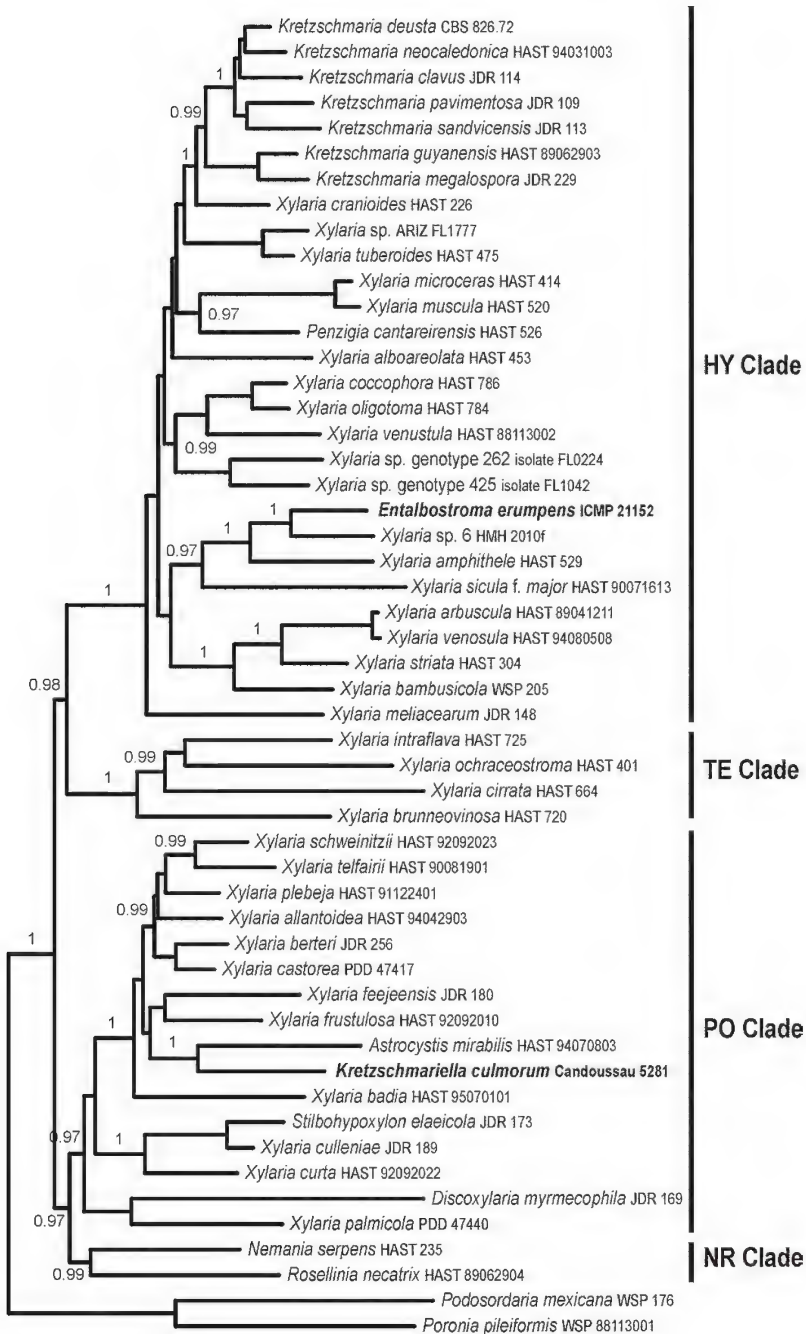
TYPE: New Zealand: Auckland: North Piha, Laird Thomson Track, on dead leaf *Phormium tenax* J.R. Forst. & G. Forst., 28 Aug 2015, coll. N.A. Martin (Holotype, PDD 107494; isotype, WSP 72775; ex type culture, ICMP 21152; GenBank KX258204, KX258205, KX258206).

ETYMOLOGY: For the erumpent nature of the ascumata from leaf tissue.

Stromata pulvinate to applanopulvinate, orbicular to elliptical to irregular, solitary to gregarious, erumpent becoming superficial, 2–10 mm long $\times$ 1–3 mm broad $\times$ 1–2 mm thick; surface blackish, interior perithecium-bearing tissue white. Perithecia more or less spherical, 0.2–0.4 mm diam. Ostioles ill defined. Asci short-stipitate, 120–146 $\times$ 8 μ m total length. Ascus apical ring inverted hat-shaped, bluing in Melzer's iodine reagent, ca. 4.5 μ m high, 2.7 μ m broad. Ascospores dark brown, ellipsoid-inequilateral, 16–19 $\times$ 8–8.8 μ m, with long germ slit on flattened side, with a hyaline gelatinous sheath that is often broader on the flattened side, and often with a small gelatinous cap at one end. Paraphysate.

Cultures from multiple ascospores on OA under 12 hr fluorescent light and 12 hr dark white to pinkish, covering 9 cm diam plate in 2–3 wk. Surface at first plane, appressed and smooth after 4–6 wk showing small areas of raised pimples. Pimples becoming enlarged and blackened, hollow. Conidiophores forming a palisade-like layer of short, cylindric cells with conidia produced holoblastically on several scars near apex. Conidia hyaline, smooth, ellipsoid with narrowed flattened bases indicating point of attachment to conidiogenous cell, (3–)4–5(–7) $\times$ 2–2.5 μ m.

FIGURE 2. Bayesian phylogenetic tree based on concatenated ITS, β -tubulin, and RPB2 sequences, Bayesian posterior probabilities >0.95 on edges. Taxa selected from U'Ren et al. (2016) are labelled with voucher numbers; *Podosordaria mexicana* and *Poronia pileiformis* were selected as outgroup. Clade designations follow U'Ren et al. (2016). *Entalbostroma erumpens* sequences are deposited in GenBank as KX258204, KX258205, and KX258206; those from *Kretzschmariella culmorum* as KX430043, KX430045, and KX430046.



ADDITIONAL SPECIMENS EXAMINED: **NEW ZEALAND: NORTHLAND:** Poor Knights Islands, on dead leaves of *Phormium tenax*, 3 Sep 1984, coll. R.E. Beever (PDD 46231). **AUCKLAND:** Piha South, Lookout Track, on dead leaves of *Phormium tenax*, 28 Aug 2015, coll. N.A. Martin (PDD 107553); Muriwai, Quarry Track, on dead leaves of *Phormium tenax*, 21 July 2015, coll. N.A. Martin (PDD 107552); Muriwai, Maori Bay Track, on dead leaves of *Phormium tenax*, 21 Aug 2015, coll. N.A. Martin (PDD 107550); Waitakere Ranges, Nihotupu Dam, on dead leaves of *Phormium tenax*, 4 Nov 1960, coll. J.M. Dingley (PDD 42824). **MID CANTERBURY:** Bealey Valley, on dead leaves of *Phormium cookianum* Le Jol. (as *P. colensoi* Hook. f.), 11 Mar 1983, coll. E. Horak (PDD 93072). **STEWART ISLAND:** Doughboy Bay, on dead leaves of *Phormium* sp., 29 Apr 2002, coll. P.R. Johnston, S. Whitton, R. Leschen (PDD 107551).

Phylogeny

The ITS sequences from the fruit body from the PDD 107494 fungarium specimen and from cultures derived from that specimen (ICMP 21152) were identical. The DNA sequences from ICMP 21152 have been deposited in GenBank as KX258204, KX258205, and KX258206.

Entalbostroma erumpens formed a strongly supported sister relationship with *Xylaria amphithele* and *Xylaria* sp. 6 sensu Hsieh et al. (2010) within the 'HY' clade of U'Ren et al. (2016) (FIG 2). Morphologically it is divergent from *X. amphithele* (San Martín González & Rogers 1989), but it shares a leaf-inhabiting ecology with both this species and *Xylaria* sp. 6 (Hsieh et al. 2010). *Kretzschmariella* is phylogenetically distant, positioned within the 'PO' clade of U'Ren et al. (2016).

Discussion

The morphology of the sexual state of *Entalbostroma* recalls *Kretzschmariella*, both genera having erumpent ascomata with a thin black crust and white soft entostromatic tissue surrounding the perithecia. The two genera differ in their anamorph, our new fungus having a reduced *Geniculosporium*-like anamorph in culture (typical of species of *Xylaria* and *Kretzschmaria*), rather than the *Mirandina*-like conidial state of *Kretzschmariella* (Ju & Rogers 1994). This reinforces earlier studies showing that the morphology of the anamorph may be phylogenetically informative among these fungi. Inclusion of morphologically divergent taxa such as *Entalbostroma* will be necessary for future phylogenetic resolution of generic level clades within the *Xylariaceae*, and for recognition of the characters that can be used to recognise those clades morphologically.

Entalbostroma erumpens is geographically widespread on dead, partly decomposed leaves of *Phormium* spp. in New Zealand, and appears to be quite common on its host.

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Yu-Ming Ju (IPMB, Academia Sinica) is thanked for providing unpublished sequences for *Kretzschmariella culmorum*. Jacques Fournier and Yu-Ming Ju are thanked for reviewing the manuscript and suggesting improvements. This research was supported through the Landcare Research Systematics Portfolio with funding from the Science and Innovation Group of the New Zealand Ministry of Business, Innovation and Employment, and by Dept. of Plant Pathology, Washington State University.

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***Podosporiopsis*, a new genus of synnematous hyphomycetes from China**

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ABSTRACT—*Podosporiopsis* gen. nov. is proposed. It is characterized by distinct unbranched conidiophores forming distinct synnematal conidiomata with emergent monotretic, integrated, terminal, determinate or percurrently extending conidiogenous cells that produce solitary, obclavate, distoseptate conidia. *Podosporiopsis sinensis* sp. nov. (the type species) and *P. obclavata* sp. nov., both collected on dead branches of unidentified plants in China, are described and illustrated. A key to *Podosporiopsis* and similar genera is provided.

KEY WORDS—anamorphic fungi, asexual morph, taxonomy

Introduction

Lushan Mountain is a world heritage site in Jiangxi Province, China, south of the Yangtze River and northwest of Poyang Lake. It covers 302 km² and is protected by an outlying buffer zone of 500 km². However, its mycobiota, especially of saprobic microfungi, are poorly known. During our continuing mycological surveys (2012–15) in this region, two novel synnematous hyphomycetes were encountered on dead branches. Close examination showed significant differences from previously described hyphomycetes, and a new genus, *Podosporiopsis*, is erected for them.

Materials & methods

At the end of rainy season, samples of dead branches were collected from humid or riparian environments, and processed as described by Ma et al.

(2012). Conidia and conidiophores were measured and photographed using a Nikon Eclipse E200 with 100× (oil immersion) objectives and SmartV550Dc digital camera. Adobe Photoshop 7.0 was used to process images into plates, with backgrounds replaced for esthetic reasons. The specimens studied are deposited in the Plant Pathology Herbarium, Jiangxi Agricultural University, Nanchang, China (HJAUP), and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS; <http://hmas.im.ac.cn>).

Taxonomy

Podosporiopsis Jian Ma, X.G. Zhang & R.F. Castañeda, **gen. nov.**

INDEX FUNGORUM IF 551862

Differs from *Podosporium* by its distoseptate conidia.

TYPE SPECIES: *Podosporiopsis sinensis* Jian Ma et al.

ETYMOLOGY: refers to its similarity to *Podosporium*.

CONIDIOMATA synnematus, solitary, erect, cylindrical, indeterminate, dematiaceous. CONIDIOPHORES synnematus, unbranched, septate, dematiaceous. CONIDIOGENOUS CELLS diverging from the synnematal axis, monotretic, integrated, terminal, determinate or with percurrent extensions. Conidial secession schizolytic. CONIDIA solitary, dry, acrogenous, distoseptate.

Podosporiopsis sinensis Jian Ma, X.G. Zhang & R.F. Castañeda, **sp. nov.** FIGS 1, 2

INDEX FUNGORUM IF 551863

Differs from *Podosporium* spp. by its distoseptate conidia.

TYPE: China, Jiangxi Province: Lushan Mountain, on dead branches of an unidentified broadleaf tree, 8 November 2014, J. Ma (**Holotype**, HJAUP M0279-1; **isotype**, HMAS 245604).

ETYMOLOGY: refers to the country in which the fungus was collected.

COLONIES effuse on the natural substratum, dark brown to black, hairy. Mycelium partly superficial, partly immersed in the substratum. CONIDIOMATA synnematus, solitary, erect, cylindrical, dark brown to black, becoming narrower toward the apex, up to 1000 µm high, 100–120 µm wide at the often swollen base, fertile region with more than half the length of the synnemata. CONIDIOPHORES distinct, synnematus, cylindrical, unbranched, septate, smooth, brown to dark brown, up to 1000 µm long, 6–10 µm wide, diverging laterally and terminally. CONIDIOGENOUS CELLS monotretic, integrated, terminal, cylindrical, smooth, brown, determinate, or sometimes with 1–2 cylindrical percurrent extensions, 12–27 × 6–8.5 µm. Conidial secession schizolytic. CONIDIA solitary, dry, acrogenous, smooth, straight or curved,

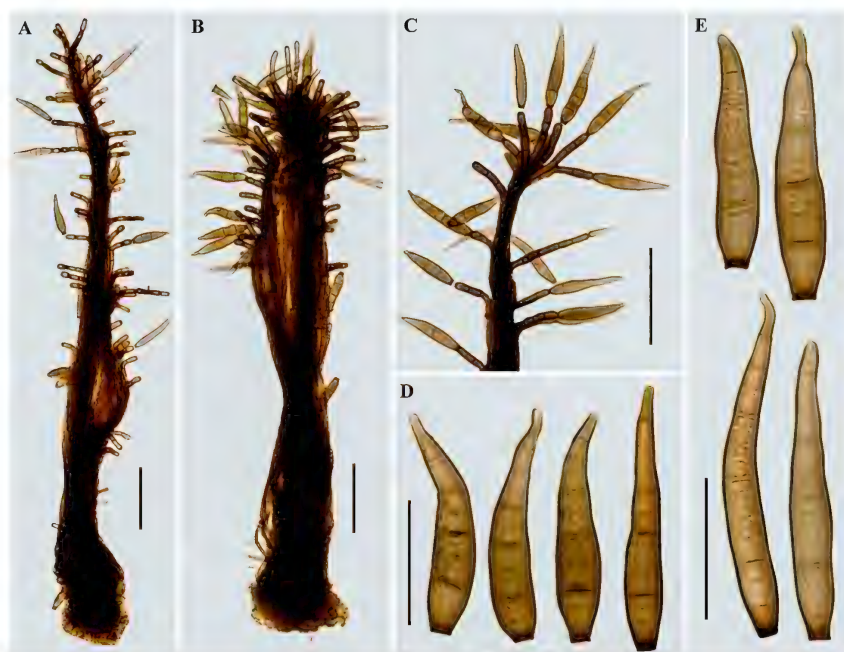


FIG. 1. *Podosporiopsis sinensis* (holotype, HJAUP M0279-1): A–C. Synnemata with conidiophores and conidia; D, E. Conidia. Scale bars: A–C = 100 µm; D, E = 40 µm.

obclavate, 10–14-distoseptate, pale brown to brown, 65–97 µm long, 10–15 µm thick at the widest part, tapering to 3–4.5 µm at the apex, 5–7 µm wide at the truncate base.

Podosporiopsis obclavata Jian Ma, X.G. Zhang & R.F. Castañeda, sp. nov. FIGS 3, 4

INDEX FUNGORUM IF 551864

Differs from *Podosporiopsis sinensis* by its longer and narrower, 8–20-distoseptate conidia with the truncate base narrower than the apex.

TYPE: China, Jiangxi Province: Lushan Mountain, on dead branches of an unidentified broadleaf tree, 8 November 2014, J. Ma (Holotype, HJAUP M0279-2; isotype, HMAS 245605).

ETYMOLOGY: *obclavata*, referring to the obclavate conidia of this fungus.

COLONIES effuse on the natural substratum, dark brown to black, hairy. Mycelium partly superficial, partly immersed in the substratum. CONIDIOMATA synnematous, solitary, erect, cylindrical, dark brown to black, becoming narrower toward the apex, ≤1420 µm tall, 65–170 µm diam. at the often



FIG. 2. *Podosporiopsis sinensis* (holotype, HJAUP M0279-1): A-C. Conidiophores with terminal, monotretic conidiogenous cells; D-F. Conidiophores with terminal conidia and 0-1 percurrent extension. Scale bars = 40 μm .

swollen base, fertile region with more than half the length of the synnemata. CONIDIOPHORES distinct, synnematus, cylindrical, unbranched, septate, smooth, brown to dark brown, $\leq 1400 \mu\text{m}$ long, 5-7.5 μm diam., diverging

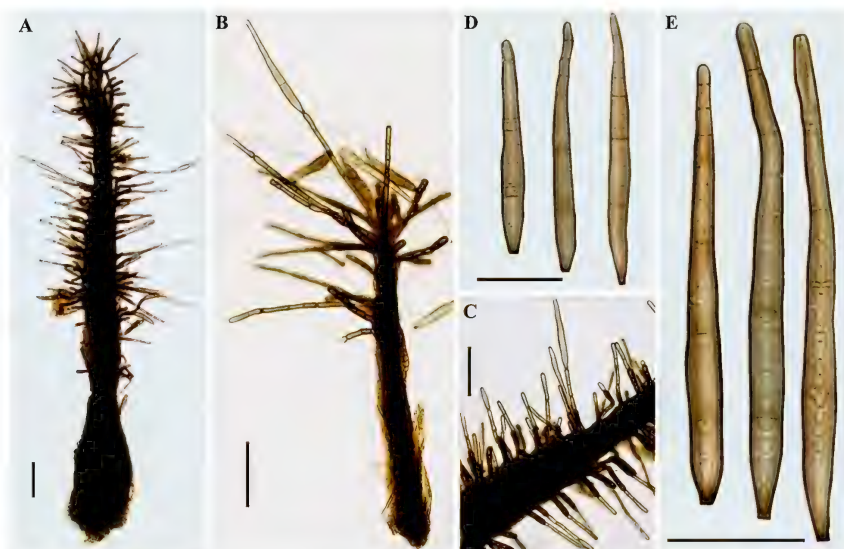


FIG. 3. *Podosporiopsis obclavata* (holotype, HJAUP M0279-2): A–C. Synnemata with conidiophores and conidia; D, E. Conidia. Scale bars: A = 100 μ m; B, C = 80 μ m; D, E = 40 μ m.

laterally and terminally. CONIDIOGENOUS CELLS monotretic, integrated, terminal, cylindrical, smooth, brown to pale brown, determinate or sometimes with 1–2 cylindrical percurrent extensions, $20\text{--}40 \times 5\text{--}6.5 \mu\text{m}$. Conidial secession schizolytic. CONIDIA solitary, dry, acrogenous, smooth, straight or curved, obclavate, 8–20-distoseptate, pale brown, $95\text{--}160 \mu\text{m}$ long, $8\text{--}11 \mu\text{m}$ diam. at the widest part, tapering to $3.5\text{--}5.5 \mu\text{m}$ at the apex and $2\text{--}4 \mu\text{m}$ at the truncate base.

Discussion

Podosporiopsis sinensis and *P. obclavata* are unique in having distinct unbranched conidiophores forming synnematosous conidiomata, and solitary obclavate distoseptate conidia seceding schizolytically from monotretic, integrated, terminal, determinate or percurrently extending conidiogenous cells diverging from the axis of the synnemata. We initially thought of assigning them to the genus *Podosporium* Schwein. (Schweinitz 1832), but the conidia in *Podosporium* are euseptate rather than distoseptate, and the addition of these species would have significantly broadened its generic concept. Furthermore, conidial septation (euseptate vs distoseptate) has often been used as a generic character to divide otherwise morphologically similar fungi (e.g., *Ellisembia* vs *Sporidesmium*, *Linkosia* vs *Stanjehughesia*, *Sporidesmiella* vs *Repetophragma*,



FIG. 4. *Podosporiopsis obclavata* (holotype, HJAUP M0279–2): A. Conidiophore with developing conidium; B, C. Conidiophores with terminal conidia, conidiogenous cells, and percurrent extensions; D. Conidiophores with terminal, monotretic conidiogenous cells and percurrent extensions. Scale bars = 40 µm.

Solicorynespora vs *Corynespora*, and *Parablastocatena* vs *Blastocatena*) and provides a narrow generic concept (Seifert et al. 2011; Zhang et al. 2012). Such distinctions also help avoid confusion and complexity in a number of hyphomycete genera and facilitate the morphological division of larger genera into smaller units (Subramanian 1992; McKenzie 1995; Wu & Zhuang 2005). For these reasons, we propose the new genus *Podosporiopsis* to accommodate *P. sinensis* and *P. obclavata*.

Podosporiopsis resembles *Dendrographium* Masee (Masee 1892), *Exosporium* Link (Link 1809), *Corynespora* Güssow (Güssow 1906), and *Solicorynespora* R.F. Castañeda & W.B. Kendr. (Castañeda-Ruíz & Kendrick 1990) in possessing distinct conidiophores with tretic conidiogenesis. However, in *Dendrographium* and *Exosporium* conidiogenesis is polytretic, in contrast to the monotretic conidiogenesis of *Podosporiopsis*. Conidiogenesis

in *Corynespora* and *Solicorynespora* is the same as in *Podosporiopsis*, but *Podosporiopsis* has synnemata conidiomata not found in *Corynespora* and *Solicorynespora*.

Several other genera including *Gangliostilbe* Subram. & Vittal (Vittal 1976), *Novozymia* W.P. Wu (Wu & Zhuang 2005), *Neosporidesmium* Mercado & J. Mena (Mercado Sierra & Mena Portales 1988), *Raizadenia* S.L. Srivast. (Srivastava 1981), and *Annellophragmia* Subram. (Subramanian 1963) have morphological characters similar to those of *Podosporiopsis*. However, conidiogenesis is polyblastic sympodial in *Annellophragmia*, monoblastic with percurrent extension in *Novozymia*, and monoblastic but producing euseptate conidia in *Gangliostilbe*, all of which differ from the monotretic conidiogenesis of *Podosporiopsis*. Conidia in *Neosporidesmium* and *Raizadenia* are distoseptate but conidiogenesis is monoblastic, an obvious difference from *Podosporiopsis*.

Key to *Podosporiopsis* and morphologically similar genera

1. Conidiogenous cells enteroblastic 2
1. Conidiogenous cells holoblastic 7
2. Conidiogenous cells polytretic 3
2. Conidiogenous cells monotretic 4
3. Conidiophores divergent from synnematal capitulum,
conidia with an non-darkened hilum *Dendrographium*
3. Conidiophores mononematous or divergent from stipe and synnematal capitulum,
conidia with a conspicuously darkened hilum *Exosporium*
4. Conidiophores mononematous 5
4. Conidiophores synnemalous 6
5. Conidia distoseptate *Corynespora*
5. Conidia euseptate *Solicorynespora*
6. Conidia distoseptate *Podosporiopsis*
6. Conidia euseptate *Podosporium*
7. Conidiogenous cells polyblastic *Annellophragmia*
7. Conidiogenous cells monoblastic 8
8. Conidiophores divergent from stipe and synnematal capitulum 9
8. Conidiophores divergent from synnematal capitulum 10
9. Conidiogenous cells with annellidic percurrent proliferation *Novozymia*
9. Conidiogenous cells determinate or with lageniform, ampulliform,
or doliiform proliferation *Neosporidesmium*
10. Conidia distoseptate *Raizadenia*
10. Conidia euseptate *Gangliostilbe*

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Distribution of *Alternaria* species among sections. 3. Sections *Infectoriae* and *Pseudoalternaria*

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ABSTRACT—Morphological examination of material conforming to the morphological description of *Alternaria* sect. *Infectoriae* (but not phylogenetically characterized) allowed the inclusion of nine additional species in this section, which thus encompasses 34 species. *Alternaria* sect. *Pseudoalternaria* consists of two species and one misidentified isolate that represents a novel taxon, described here as *Alternaria parvicaespitosa*. Emended descriptions of *A.* sect. *Infectoriae* and *A.* sect. *Pseudoalternaria* are presented, and their species are listed.

KEY WORDS—*Alternaria infectoria*, *Alternaria rosae*, *Lewia*

This article is dedicated to the jubilee of the flourishing 200-year-old girl, *Alternaria*.

Introduction

Alternaria Nees 1816 is a large pleomorphic genus that comprises approximately 280 distinguishable species (Simmons 2007). A large series of morphological and phylogenetic studies have attempted to better resolve the phylogeny of *Alternaria* and other alternarioid hyphomycetes by utilizing more than ten different genomic loci (Pryor & Bigelow 2003; Hong et al. 2005; Runa et al. 2009; Lawrence et al. 2012, 2013, 2014; Woudenberg et al. 2013, 2014; Armitage et al. 2015). Several well-supported lineages (phylogenetic species-groups) have been revealed within the alternarioid hyphomycetes and have led to several taxonomic novelties. The genus *Alternaria* was recently divided into eight taxonomic sections by Lawrence et al. (2013) followed by the elevation

of 19 additional clades by Woudenberg et al. (2013, 2014), Grum-Grzhimaylo et al. (2016), and Lawrence et al. (2016), thereby bringing the total number of sections of *Alternaria* to 27.

All works support the monophyly of two morphologically peculiar *Alternaria* groups, two of several groups having members with catenate, small-spored conidia. Recently these groups were elevated to sectional status, as *A. sect. Infectoriae* and *A. sect. Pseudoalternaria*.

Twenty-five species were indicated as members of sect. *Infectoriae* by Lawrence et al. (2013). Woudenberg et al. (2013) added two additional species to this clade, bringing the total number of recognized species to 27. Lawrence et al. (2013) also mentioned three species (*A. peglionii*, *A. dianthicola*, and *A. photistica*) that cannot be included in sect. *Infectoriae*. The sequenced strain of *A. peglionii* (CBS 103.26) was not the type, and this species was previously listed as *incertae sedis* (Simmons 2007). The sequenced strain of *A. dianthicola* was not designated by E.G. Simmons as a representative strain. When a representative strain of *A. dianthicola* was utilized it clustered distantly to sect. *Infectoriae* (Woudenberg et al. 2013). Also Lawrence et al. (2013) and Woudenberg et al. (2013) used the same strain of *A. photistica* (EGS 35.172), but it was obtained from different sources. Conclusions by Woudenberg et al. (2013) based on molecular and morphological data placed *A. photistica* in sect. *Panax* and *A. dianthicola* in sect. *Dianthicola*. Ariyawansa et al. (2015) described a new species, *A. murispora*, in section *Infectoriae* based on ascomatal morphology and DNA analysis; no living culture or conidial state of this species is known. Ariyawansa et al. (2015) also noted that the type isolate of *Xenobotryosphaeria calamagrostidis* (CBS 303.71) clustered in sect. *Infectoriae* (albeit with low support) and only the sexual state was produced in culture and clearly differed from species within *Alternaria* sect. *Infectoriae*. The authenticity of *X. calamagrostidis* remains unresolved and would benefit from a re-examination of morphological and molecular characters for this peculiar isolate/taxon.

The number of species recovered by phylogenetic species recognition approaches can differ from that revealed by morphological analysis. Very likely a morphological concept favors splitting. For instance, phylogenetic analyses of three genomic regions from 39 infectoria species-group isolates (Andersen et al. 2009) were unable to provide strong support for species-level designations for most isolates. No correlations were found among phylogenetic data, metabolite production, morphological identity, proascoma formation, ability to grow at 37°C, and host or geographic origin. Gannibal & Yli-Mattila (2007), who utilized a set of 52 infectoria species-group isolates (including representative strains of eight morphospecies), were unable to divide the isolates into distinct groups based on data from molecular fingerprinting.

The main discriminating morphological features of many *Alternaria* sect. *Infectoriae* species are small conidia (in culture usually no longer than 60 µm) and long secondary conidiophores (Lawrence et al. 2013; Woudenberg et al. 2013). Ten species have a known sexual state (*A. alternarina*, *A. daucicaulis*, *A. ethzedia*, *A. hordeiaustralica*, *A. hordeicola*, *A. infectoria*, *A. intercepta*, *A. murispora*, *A. scrophulariae*, and *A. viburni*). Some strains are capable of producing proascoma in axenic culture, suggesting that members of this group, at least in part, are homothallic (Andersen et al. 2009). In general fungi in this section are common. Usually they exist as saprobes showing no substrate preference. This group produces reliable chemotaxonomic markers (Christensen et al. 2005; Andersen et al. 2002, 2009). Some authors have shown that strains of this group do not produce mycotoxins generally made by other small-spored *Alternaria* sections (e.g., sect. *Alternaria*) (Andersen et al. 2002). Other studies revealed the ability of some species to produce alternariol and monomethyl ether (Oviedo et al. 2013), which probably is common for other *Alternaria* sections.

Lawrence et al. (2014) assigned two species, sister to *A.* sect. *Infectoriae*, to a new genus, “*Pseudoalternaria*” nom. inval. This placement became untenable after all alternarioid hyphomycetes were collected under the single generic name *Alternaria* (Woudenberg et al. 2013). The thorough review by Lawrence et al. (2016) resurrected “*Pseudoalternaria*” as a new section, *Alternaria* sect. *Pseudoalternaria*, typified by a new species, *Alternaria arrhenatheri*.

Section *Pseudoalternaria* currently consists of two described species, *A. arrhenatheri* D.P. Lawr. et al. and *A. rosae* E.G. Simmons & C.F. Hill, and one misidentified isolate (X1272, isolated from blueberry and identified as *A. rosae* by Zhu & Xiao 2015) but which represents a third/new species (based on molecular and morphological data), described here as *A. parvicaespitosa*.

Ongoing molecular studies will likely identify additional species and novel lineages within *Alternaria*. However, living cultures are not known for several morphospecies. Additionally some herbaria materials are recalcitrant to DNA isolation and/or devoid of conidia. Fortunately, the morphological diversity of *Alternaria* has been well studied (Simmons 2007). Classical comparative morphological studies are useful for establishing phylogenetic affiliation. This work is a continuation of the series started with the manuscripts on sections *Porri* and *Alternaria* (Gannibal 2015, 2016). The aim of the present work is to detect among phylogenetically unexamined *Alternaria* species those species that fit the current morphological circumscription of sections *Infectoriae* and *Pseudoalternaria* and to emend the morphological concept of these sections.

Materials & methods

The morphology of almost all *Alternaria* species with legitimate names was analyzed with regard to conformity with criteria of *Alternaria* sections *Infectoriae* and *Pseudoalternaria*. The morphological assessment was based on descriptions made by Simmons (2007) or using original diagnoses and illustrations for species described after 2007. Descriptions of three species included in sect. *Infectoriae* were found in Roberts (2008: *A. roseogrisea*), Roberts et al. (2010: *A. cerasidanica*), and Ariyawansa et al. (2015: *A. murispora*). Strain X1272 was cultured on potato carrot agar (PCA) at 24°C under light/dark cycle (12/12 h) for 7–10 days to promote sporulation in order to describe this as a new taxon; the holotype specimen (a metabolically inactive dried PCA culture) was conserved in the Mycological Herbarium, All-Russian Institute of Plant Protection, Saint Petersburg, Russia (LEP).

Results & discussion

The evaluation of all available descriptions of *Alternaria* species allowed for the inclusion of nine additional species in sect. *Infectoriae*, two of which have no known living isolates. The diversity of this section consists of 34 morphologically recognizable species. Recircumscription of the misidentified isolate, *A. rosae* strain X1272 (Zhu & Xiao 2015), allowed for the addition of one species to sect. *Pseudoalternaria* bringing the total number of species in this clade to three.

Alternaria parvicaespitosa Gannibal & D.P. Lawr., sp. nov.

FIGS 1, 2

MYCOBANK MB 816666

Differs from *Alternaria rosae* by its shorter conidial chains (non-branched parts) and narrower conidia with fewer transverse septa.

TYPE: USA, California, Tulare, harvested blueberry fruit from a commercial fruit-packing center, 6 June 2013, X.Q. Zhu & C.L. Xiao X1272 (Holotype, LEP 014858, a metabolically inactive dried culture).

ETYMOLOGY: *parvi* (Latin), small; *caespitosa* (Latin), bushy.

Sporulation on PCA occurs primarily in discrete bushy clumps. Conidiophores arise as series in close proximity to one another from submerged or (more often) aerial hyphae. Aerial conidiogenous hyphae are hyaline filiform or thick colored conidiophore-like and may be positioned vertically or horizontally. Most conidiophores remain relatively short, 35–70 × 3–4 µm. They are simple or sometimes produce two branches. Each branch is multigeniculate (1–6 genua near conidiogenous loci). The conidiogenous sites produce short chains of conidia (≤3–4 between branching points) which usually have one or a few branches due to the production of several conidiogenous sites on an apical secondary conidiophore or (very rarely) due to the formation of a lateral secondary conidiophore. Aged clumps of conidia developed on a single conidiophore may comprise ≤20 conidia.

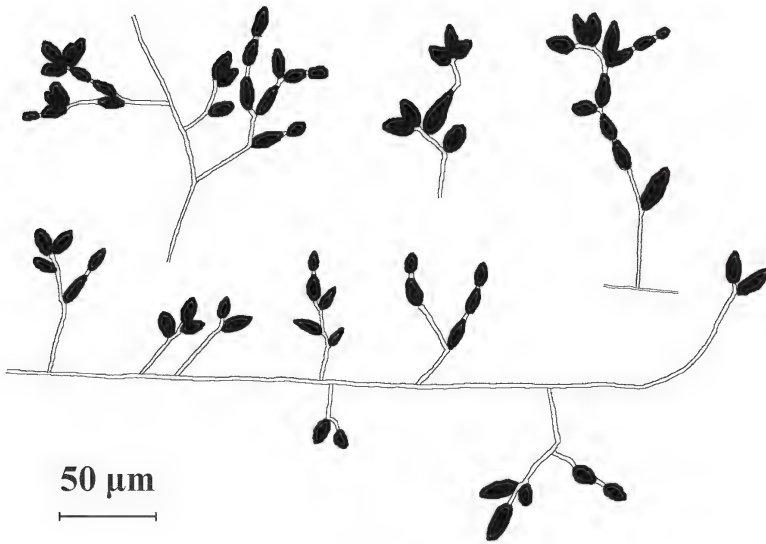


FIG. 1. *Alternaria parvicaespitosa* (ex holotype, LEP 014858): sporulation pattern.

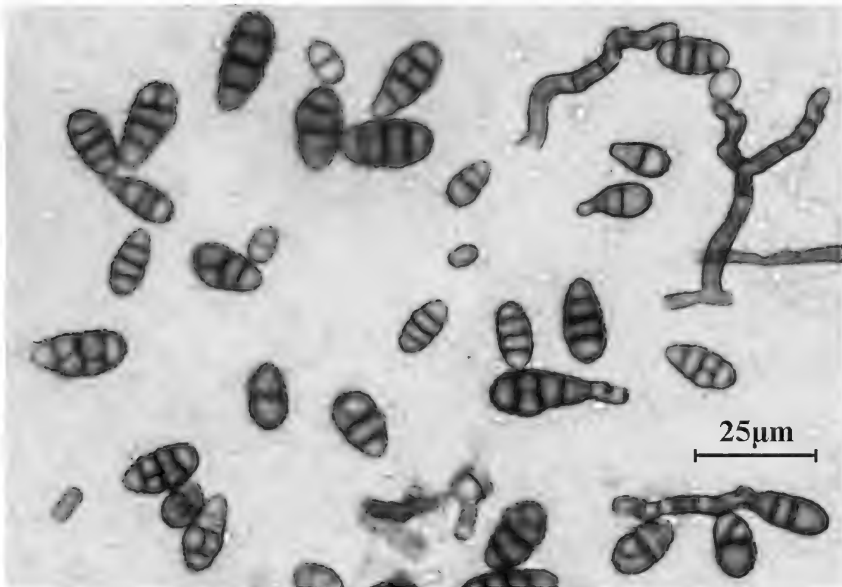


FIG. 2. *Alternaria parvicaespitosa* (ex holotype, LEP 014858): conidia and conidiophores.

Both juvenile and mature conidia are ovoid or elliptical. Small portion of conidia is obclavate. Maximum size range of mature conidia is $10\text{--}25 \times 7\text{--}12\text{ }\mu\text{m}$. Conidia have 1–3(–4) transverse septa and at most one longiseptum in 1–2 of the transverse segments. Rarely conidia have no longisepta. Most conidia have no secondary conidiophores; but any conidium, including 1-celled units and conidia with relatively large conidial bodies, can produce an apical secondary conidiophore (a single cell ca $3 \times 3\text{ }\mu\text{m}$) or a geniculate extension $5\text{--}28\text{ }\mu\text{m}$ long with 2–5 conidiogenous sites. Rarely apical secondary conidiophores are branched. Lateral secondary conidiophores appear rarely and when present are short. Conidia and conidiophores are concolorous, medium brown. The conidium outer wall is smooth or (in the largest conidia) slightly punctulate.

Alternaria species in sections *Infectoriae* and *Pseudoalternaria* are listed in TABLE 1.

Herein we present emended and somewhat expanded versions of the descriptions of *Alternaria* sect. *Infectoriae* by Woudenberg et al. (2013) and of *A.* sect. *Pseudoalternaria* by Lawrence et al. (2016). Characters are quantified when appropriate, and examples are provided of species whose quantified characters either depart from the section norm or conform to it.

Alternaria sect. *Infectoriae* Woudenb. & Crous, Stud. Mycol. 75: 194. 2013; emend. Gannibal & Lawrence

On PCA or weak potato dextrose agar (WPDA) primary conidiophores arise from the agar surface or from vegetative aerial hyphae. Primary conidiophores are solitary or aggregated, cylindrically straight or geniculate, simple or branched, short to long, septate, brown, $20\text{--}150 \times 3\text{--}4\text{ }\mu\text{m}$, with one or several (2–6) conidiogenous loci (pores) at the apex and throughout its length. Conidial chains are moderately long to long, simple or (usually) branched. Branching is produced by the occurrence of several conidiogenous loci on apical secondary conidiophores or (rarely: in some species completely absent) lateral secondary conidiophores.

Young conidia are ovoid, broadly ovoid, ellipsoid or subellipsoid, erostrate or with short to long secondary conidiophore at the apex. Mature conidia are obclavate, ovoid, ellipsoid or subellipsoid; small or moderate in size; with (1–)4–7(–10) transverse and no, one or a few longitudinal septa; slightly constricted near some transverse septa. Size of conidia is $30\text{--}60 \times 6\text{--}15\text{ }\mu\text{m}$ (except conidia with long secondary conidiophores); sometimes a large number of conidia remain small ($12\text{--}30 \times 5\text{--}10\text{ }\mu\text{m}$). Several species (e.g., *A. trititina*) can produce relatively larger conidia, $\leq 60 \times 25\text{ }\mu\text{m}$, not including secondary

TABLE 1. List of *Alternaria* spp. assigned to *A. sect. Infectoriae* and *A. sect. Pseudoalternaria*.

Names with bracketed annotations = species with phylogenetic data (in the cited references).

Unannotated names = species with reliable living cultures and herbarium specimens.

Names with asterisk = species for which no known living isolates are available.

Section *Infectoriae*

- A. alternarina* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. arbusti* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. astragali* Wangeline & E.G. Simmons
- A. caespitosa* (de Hoog & C. Rubio) Woudenb. & Crous (Woudenberg et al. 2013; Grum-Grzhimaylo et al. 2015)
- A. californica* E.G. Simmons & S.T. Koike (Lawrence et al. 2013, 2014)
- A. cerasi* Potebnia [*]
- A. cerasidanica* R.G. Roberts
- A. daucicaulis* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. ethzedia* E.G. Simmons (Pryor & Bigelow 2003; Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013; Grum-Grzhimaylo et al. 2015)
- A. frumenti* E.G. Simmons & C.F. Hill (Lawrence et al. 2013, 2014)
- A. geophila* Dasz.
- A. graminicola* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. hordeiaustralis* E.G. Simmons & Alcorn (Lawrence et al. 2013, 2014)
- A. hordeicola* E.G. Simmons & Kosiak (Lawrence et al. 2013, 2014)
- A. humuli* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. incomplexa* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. infectoria* E.G. Simmons (Pryor & Bigelow 2003; Lawrence et al. 2013; Woudenberg et al. 2013; Grum-Grzhimaylo et al. 2015)
- A. intercepta* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. iridiaustralis* E.G. Simmons, Alcorn & C.F. Hill
- A. litorea* (Pivkin & Zvereva) E.G. Simmons [*]
- A. merytae* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. metachromatica* E.G. Simmons (Hong et al. 2005; Lawrence et al. 2013, 2014)
- A. murispora* Ariyaw. & K.D. Hyde [*] (Ariyawansa et al. 2015)
- A. novae-zelandiae* E.G. Simmons (Lawrence et al. 2013, 2014)
- A. oregonensis* E.G. Simmons (Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013; Grum-Grzhimaylo et al. 2015)
- A. roseogrisea* R.G. Roberts
- A. scrophulariae* (Desm.) Rossman & W.C. Allen (Hong et al. 2005; Lawrence et al. 2013; Woudenberg et al. 2013; Grum-Grzhimaylo et al. 2015)
- A. seleniiphila* Wangeline & E.G. Simmons
- A. slovaca* (Svob.-Pol., L. Chmel & Bojan.) Woudenb. & Crous (Woudenberg et al. 2013; Grum-Grzhimaylo et al. 2015)
- A. triticimaculans* E.G. Simmons & Perelló (Lawrence et al. 2013, 2014)
- A. triticina* Prasada & Prabhu (Pryor & Bigelow 2003; Lawrence et al. 2013)
- A. vaccinii* E.G. Simmons
- A. ventricosa* R.G. Roberts (Lawrence et al. 2013, 2014)
- A. viburni* E.G. Simmons (Lawrence et al. 2013, 2014)

Section *Pseudoalternaria*

- A. arrhenatheri* D.P. Lawr., Rotondo & Gannibal (Lawrence et al. 2014, 2016)
- A. rosae* E.G. Simmons & C.F. Hill (Lawrence et al. 2013, 2014)
- A. parvicaespitosa* Gannibal & D.P. Lawr. (Zhu & Xiao 2015, as "*A. rosae*")

conidiophores. Some mature conidia remain erostrate or produce secondary conidiophores. Apical secondary conidiophores are short or long, simple or branched, usually geniculate with one or (often) a few ($\leq 5-7$) conidiogenous loci over its entire length. Approximately 30% of mature conidia produce at least 4 transverse septa in the conidium body terminating in a chain that is ovoid or ellipsoid and has no secondary conidiophores, beak or conidiogenous loci on apical cell. At least 10% of mature conidia produce apical secondary conidiophores that are longer than conidial body and exceed 30 μm (often reaching 50–100+ μm). Long apical conidiophores having only one transverse septum and no longitudinal septa are sometimes produced by very small conidia. Some conidia have short solitary lateral secondary conidiophores with one or two conidiogenous loci. Conidia are pale brown, yellowish to dark brown; the spore wall is smooth or conspicuously ornamented.

Ascomata on natural substrata are erumpent, almost superficial or submerged into host cortex, scattered, subglobose to sphaeroid with a short blunt beak, dark, smooth, thin-walled at maturity. Asci subcylindrical, straight, curved or muriform, usually 8-spored. Mature ascospores are ellipsoid, 5–7 transverse septa, forming one or two longitudinal septa through each central or sometimes through apical transverse segments, conspicuously constricted at median septum and slightly so at two other transepta, brown.

Colony color on rich media like PDA, PSA, DRYES or Czapek-Dox agar, is light: white, delicate rose, light gray or pale olivaceous. Colonies usually do not sporulate in the dark, especially on rich media. Sporulation is usually copious on PCA or V8 agar medium, intensive light or near UV light (when grown in glass Petri dishes).

Alternaria sect. *Pseudoalternaria* D.P. Lawr., Rotondo & Gannibal, Mycol. Progr. 15:3 [11 of 22]. 2016; emend. Gannibal & Lawrence

On PCA or WPDA sporulation aggregated, appearing granular to the naked eye. Mycelium subhyaline to light brown; hyphae smooth, branched, septate, 3.8–5 μm wide. Primary conidiophores aggregated on agar surface or developing from arachnoid aerial hyphae, simple or branched, septate, smooth, medium brown, 8.7–37.5 $\times$ 3.7–5 μm ($M = 18.1 \times 4.8 \mu\text{m}$), simple with single apical pore. Secondary conidiophores short to long, simple to multi-geniculate with one to many conidiogenous loci. Conidia 10–32.5 $\times$ 4.7–10 μm ($M = 16.8 \times 7.3 \mu\text{m}$), mostly catenulate, ellipsoid to obclavate, medium brown to golden brown, 3–4 transverse septa, 1–2 longitudinal septa, smooth, may produce a secondary conidiophore (false beak).

Acknowledgments

The authors are grateful to Dr. Hiran Ariyawansa (Guizhou Academy of Agricultural Sciences, Guizhou University, Guiyang, China) and Dr. Andrew Armitage (Genetics and Crop Improvement, East Mailing Research, UK) for presubmission review. This work was supported by Russian Science Foundation (project #14-26-00067).

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***Zasmidium oleae* sp. nov. from China**

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ABSTRACT—A new species, *Zasmidium oleae* on *Olea tsoongii* is described, illustrated and discussed. The type specimen is deposited in HMAS.

KEY WORDS—cercosporoid fungi, *Mycosphaerellaceae*, plant pathogen

Introduction

The genus *Zasmidium*, established by Fries (1849), was considered a monotypic genus until recently. Based on molecular sequence analyses, this genus is now used for cercosporoid fungi in the *Mycosphaerellaceae* morphologically characterized by verruculose superficial hyphae in vivo (rarely lacking); smooth to verruculose conidiophores; conspicuous, somewhat thickened and darkened, planate conidiogenous loci; and solitary or catenate conidia with thin to somewhat thickened, smooth to verruculose walls and somewhat thickened and darkened planate hila (Braun et al. 2013). *Zasmidium oleae*, a new species on *Olea tsoongii* collected in Hainan Province, China, is described, illustrated, and compared with other cercosporoid species on *Oleaceae*. The type specimen is deposited in the Herbarium Mycologium, Chinese Academy of Sciences, Beijing, China (HMAS).

Taxonomy

Zasmidium oleae Y.L. Guo, X.W. Xie & B.J. Li, **sp. nov.**

FIG. 1

FUNGAL NAME FN570159

Differs from *Passalora amurensis* by its shorter, smooth, 3-4-septate conidia.

[#]These authors contributed equally to this work

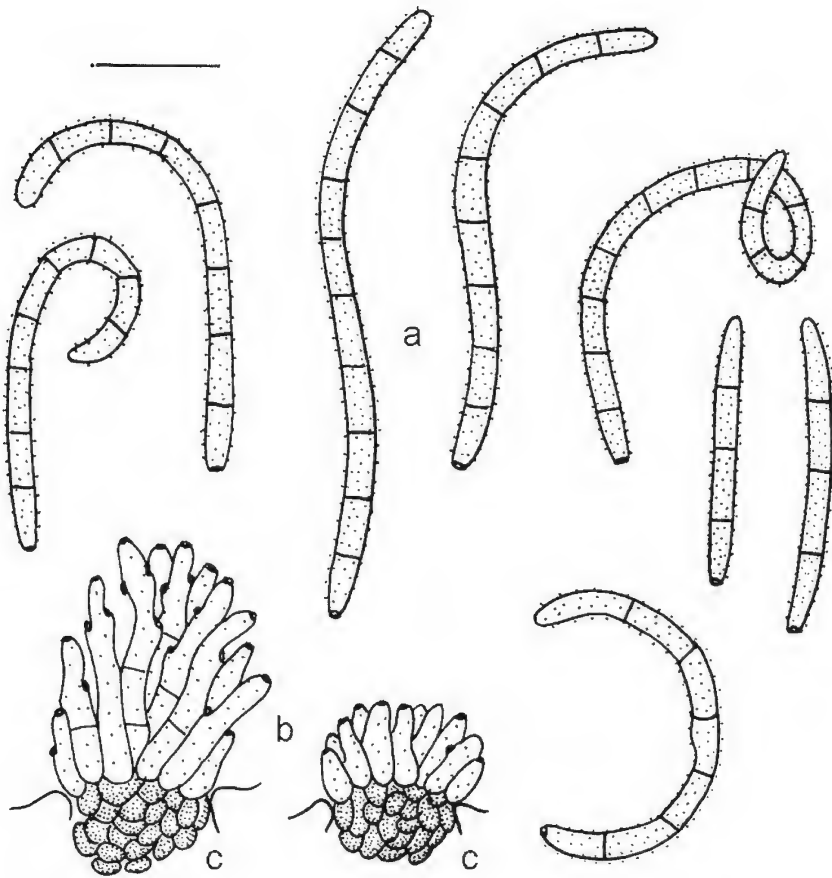


FIG. 1. *Zasmidium oleae* (HMAS 245707, holotype).
a. Conidia; b. Conidiophores; c. Stroma. Bar = 25 μ m.

TYPE: China, Hainan Province, Wanning, on living leaves of *Olea tsoongii* (Merr.) P.S. Green (*Oleaceae*), 10 April 2011, coll. Guo Ying-lan 380 (Holotype, HMAS 245707).

ETYMOLOGY: Derived from the host genus *Olea*.

Leaf spots amphigenous, circular to angular, 2–8 mm diam., often confluent, red-brown to dark brown with a yellow to pale olivaceous-brown halo on the upper surface, pale brown with yellow to pale olivaceous-brown halo on the lower surface. Caespituli mainly hypogenous. Mycelium internal. Stromata

substomatal, globose, pale brown to brown, 25–50 µm diam. Conidiophores densely fasciculate, pale olivaceous-brown to olivaceous-brown, uniform in color, irregular in width, straight to slightly curved, not branched, geniculate, thin-walled, smooth, conical to conically truncate at the apex, 0–2-septate, mostly aseptate, $6.7\text{--}20(-48) \times 4\text{--}5.3$ µm. Conidiogenous cells integrated, terminal or mostly reduced to conidiogenous cells, 6.7–20 µm long. Conidiogenous loci conspicuous, thickened, darkened, 1.3–2.5 µm wide. Conidia formed singly, cylindrical, brown, thick-walled, verruculose, straight to variously curved, rounded at the apex, rounded to short obconically truncate at the base, indistinctly 3- to multiseptate, $40\text{--}128 \times 4.0\text{--}5.8$ µm, hila thickened and darkened, 1.3–2.5 µm wide.

Discussion

Based on the description in Chupp (1954), *Cercospora lumbricoides* Turconi & Maffei on *Fraxinus* sp. (*Oleaceae*) is very similar to *Z. oleae* but differs by its brown, longer conidiophores ($30\text{--}60 \times 4\text{--}6$ µm) and longer conidia ($80\text{--}200 \times 4\text{--}6$ µm). However, the generic affinity of *C. lumbricoides* is quite unclear (the type material has not been traced and the species is known only from the original description) (Turconi & Maffei 1915).

Passalora amurensis (Ziling) H.D. Shin & U. Braun on *Syringa amurensis* Rupr. (*Oleaceae*), also morphologically similar to *Z. oleae*, differs in having pale olivaceous, 3–4-septate, shorter, and smooth conidia ($35\text{--}70 \times 4.0\text{--}5.5$ µm; Shin & Braun 1996).

Although superficial verruculose mycelium, common in most *Zasmidium* species, is lacking on *Olea tsoongii*, assignment of the new species to *Zasmidium* is nevertheless justified. Several comparable species with distinctly verruculose conidia have recently been allocated to *Zasmidium* based on molecular data that support their affinities to this genus.

Acknowledgments

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***Strigula sinoaustralis* sp. nov. and three *Strigula* spp. new to China**

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ABSTRACT—A new foliicolous lichen is described from South China. *Strigula sinoaustralis* is most similar to *S. concreta* in ascospore dimensions but differs by its white-punctate thallus with entire margins and its longer asci. An analysis of its relationships based on molecular phylogeny is given. *Strigula antillarum*, *S. laureriformis*, and *S. prasina* are reported as new to China.

KEY WORDS—new species, taxonomy, phylogenetic analysis, lichenized *Ascomycota*

Introduction

Strigula Fr. (*Strigulaceae*) is a genus of lichenized fungi containing about 70 species (Hyde et al. 2013). Seventeen *Strigula* species (twelve foliicolous; five corticolous or saxicolous) have been recorded from China (Santesson 1952, Wei & Jiang 1991, Aptroot & Seaward 1999, Aptroot & Sipman 2001, Aptroot 2003, Aptroot et al. 2003, Lücking 2008). During our ongoing studies of lichenized fungi in China, we found material representing an undescribed *Strigula* species and three *Strigula* species new to China, which are reported here.

Materials & methods

Lichen collections

Most *Strigula* specimens in this study were collected from Guangxi, Hainan, Yunnan, and Guangdong provinces and are preserved in the Fungarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS-L); one additional collection from Xizang is preserved in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN).

Phenotypic analysis

A Leica M125 dissecting microscope was used for the morphological studies and a Zeiss Axioscope2 compound microscope with a Zeiss Axio Imager A2 for the anatomical studies. Sections were studied in tap water and photographed with an AxioCam MRc5 camera. Thin-layer chromatography (TLC) (Culberson & Kristinsson 1970, Culberson 1972, Orange et al. 2001) was used to detect lichen substances.

TABLE 1. Specimens of *Strigula* spp. and *Falciformispora* outgroup used in the phylogenetic analysis

SPECIES	VOUCHER SPECIMEN	GENBANK No.
<i>S. antillarum</i>	HMAS-L 0137209	KX216696*
	HMAS-L 0137208	KX216697*
	HMAS-L 0137211	KX216702*
<i>S. prasina</i>	HMAS-L 0137213	KX216700*
	HMAS-L 0137212	KX216701*
<i>S. smaragdula</i>	KoLRI016344	KF553663
	LFF016344	KF553664
<i>S. sinoaustralis</i>	HMAS-L0137203 (T)	KX216699*
	HMAS-L0137204	KX216698*
<i>F. senegalensis</i>	IP614.60	KP132365
<i>F. tompkinsii</i>	IP559.60	KP132366

* = sequences newly generated for this study by the authors

DNA extraction, amplification, and sequencing

Seven fresh specimens representing three different *Strigula* species reported from China were chosen for DNA extraction (TABLE 1). The extraction procedure followed a modified CTAB method (Rogers & Bendich 1988). The nuclear ribosomal RNA gene region, including the internal transcribed spacer (ITS), was amplified through PCR using the primer pair ITS5 and ITS4 (White et al. 1990). Reactions were carried out in 25 µl reaction volume including 1 µl DNA, 1 µl each primer (10 µM), 2 µl dNTP (2.5 mM each), 2.5 µl amplification buffer (10×, 25 mM MgCl₂ contained), 0.5 µl Taq polymerase (rTaq DNA Polymerase, 5 U/µl), 17 µl ddH₂O. Cycling parameters were set to an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 50 s, and a final extension at 72°C for 10 min. The new sequences generated for this study were deposited in GenBank (TABLE 1).

Phylogenetic analysis

Eleven sequences including seven sequences were newly generated for this study (TABLE 1) were aligned with the program MAFFT (Katoh 2002); two other accessions of *Strigula smaragdula* Fr. were retrieved from GenBank (Jayalal et al. 2013). Due to lack of ITS sequences of other genera in *Strigulales*, two species, *Falciformispora tompkinsii* (El-Ani) S.A. Ahmed et al. and *Falciformispora senegalensis* (Segretain et al.) S.A. Ahmed et al., belonging to *Pleosporales* (the family most closely related to *Strigulales*), were chosen

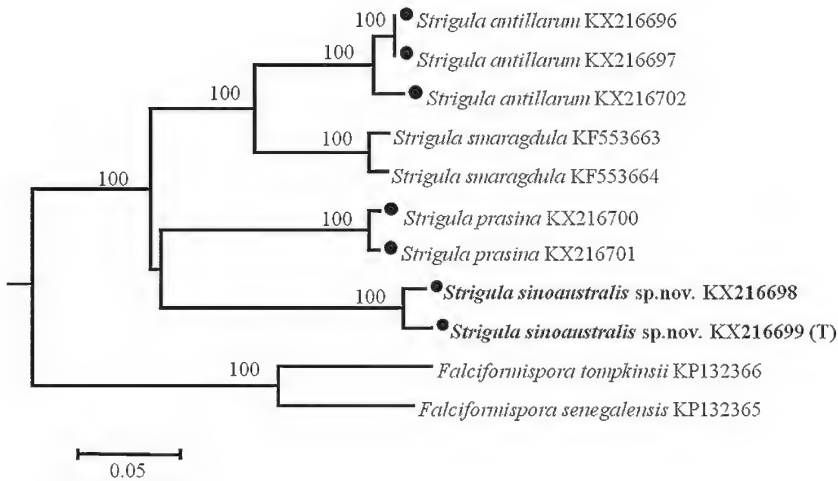


FIG. 1. The Neighbor-Joining tree based on nrDNA ITS sequences. Samples marked with '●' were sequenced by the authors; others were downloaded from GenBank. Genetic distance scale = 0.05 changes per site. Numbers above each node represents bootstrap support values >50%.

as outgroup. The alignment was subjected to a Neighbor-Joining analysis involving 1000 replicates with MEGA (Tamura et al. 2011), using the p-distance model.

Results

The best-scoring Neighbor-Joining tree, based on the eleven ITS sequences (481 bp), is shown in FIG. 1. Within the well-supported (bootstrap = 100%) monophyletic four-species clade of *Strigula*, the new species *S. sinoaustralis* and the Chinese material of *S. antillarum* and *S. prasina* each formed a separate well-supported branch (bootstraps = 100%).

Taxonomy

Strigula sinoaustralis S.H. Jiang, X.L. Wei & J.C. Wei, sp. nov.

PL. 1

FUNGAL NAMES FN570267

Differs from *Strigula concreta* by its white-punctate thallus with entire margins and its longer asci.

TYPE: China. Guangxi Province, Shangsi County, Shiwan mountain forest park, along the way to Yingkesong and Jiulong song, 21°54'16"N 107°54'09"E, alt. 271 m, on living leaves, 26/V/2015, Xin-Li Wei & Jiao-Hong Wang GX20150342 (Holotype, HMAS-L 0137203; GenBank KX216699).

ETYMOLOGY: The epithet *sinoaustralis* refers to South China.

THALLUS subcuticular, dispersed into rounded patches with entire margins, without forming distinct lobes, 2–11 mm across and 25–35 μm thick; surface smooth, grayish green, often minutely white-punctate. PHOTOBIONT a *Cephaleuros* sp., composed of angular-rounded cells, $8\text{--}14 \times 4\text{--}6 \mu\text{m}$.

PERITHECIA exposed, almost conical, basal part somewhat broadly spreading, 0.25–0.4 mm diam. and 100–180 μm tall, black. EXCIPULUM prosoplectenchymatous, 10–18 μm thick, brown; INVOLUCRELLUM 22–40 μm thick, carbonaceous, black; PARAPHYSES unbranched. ASCI cylindrical or almost thread like, numerous and dense, $72.5\text{--}92.5 \times 4\text{--}5 \mu\text{m}$. ASCOSPORES 8 per ascus, uniseriate, ellipsoid, 1-septate, usually with 1–2 oil droplets per cell when fresh, distinctly constricted at the septum and sometimes broken into parts (fragmented) outside the asci, $8\text{--}12 \times 2.5\text{--}3 \mu\text{m}$ (4–6 μm long for each fragment), 3–4 times as long as broad.

PYCNIDIA usually rather few, wart-shaped, 0.05–0.15 mm diam.; two types present: one type 0.1–0.15 mm diam. producing macroconidia that are bacillar, 0–1-septate, $4\text{--}6 \times 1.8\text{--}2.3 \mu\text{m}$, with a mucilaginous appendage at one or both ends, 5–10 μm long; the second type 0.05–0.1 mm diam., rather few, and often empty. MICROCONIDIA not seen.

CHEMISTRY. No substances detected by TLC.

ECOLOGY & SUBSTRATE. Found in humid, partly exposed habitats on living leaves of bamboo.

ADDITIONAL SPECIMENS EXAMINED: CHINA. GUANGDONG PROVINCE, Shixing County, Chebaling national nature reserve, along the way to Xianrendong Village, $24^{\circ}44'7''\text{N}$ $114^{\circ}12'31''\text{E}$, alt. 486 m, on living leaves, 15/V/2015, X.L. Wei & J.H. Wang GD2015042-8 (HMAS-L 0137206). GUANGXI PROVINCE, Shangsi County, Shiwan mountain forest park, along the Shitou River, $21^{\circ}54'3''\text{N}$ $107^{\circ}54'26''\text{E}$, alt. 342 m, on living leaves, 25/V/2015, X.L. Wei & J.H. Wang GX20150265 (HMAS-L 0137204; GenBank KX216698), GX20150387 (HMAS-L 0137205).

REMARKS: Two other *Strigula* species with carbonized perithecia develop similarly white-punctate thalli: *S. lacericola* P.M. McCarthy, which can be distinguished by its shallowly lobate thallus margins and very narrow (5–7 times longer than broad) ascospores that do not separate either within or outside the asci (McCarthy 2009) and *S. albomaculata* Sérus., which has longer asci ($120\text{--}140 \times 5\text{--}8 \mu\text{m}$) and larger ascospores ($18\text{--}22 \times 5\text{--}6 \mu\text{m}$) and macroconidia ($8\text{--}8.5 \times 1.5\text{--}2 \mu\text{m}$) (Aptroot et al. 1997).

Anatomically, the new species is most closely related to *S. concreta* (Fée) R. Sant. Both share the same perithecial anatomy and small, uniseriate ascospores that fragment outside the asci, but they differ in thallus morphology and ascus dimensions: *S. concreta* lacks white dots on the thallus and has lobulate thallus margins and shorter asci ($30\text{--}70 \times 4\text{--}6 \mu\text{m}$; Santesson 1952).

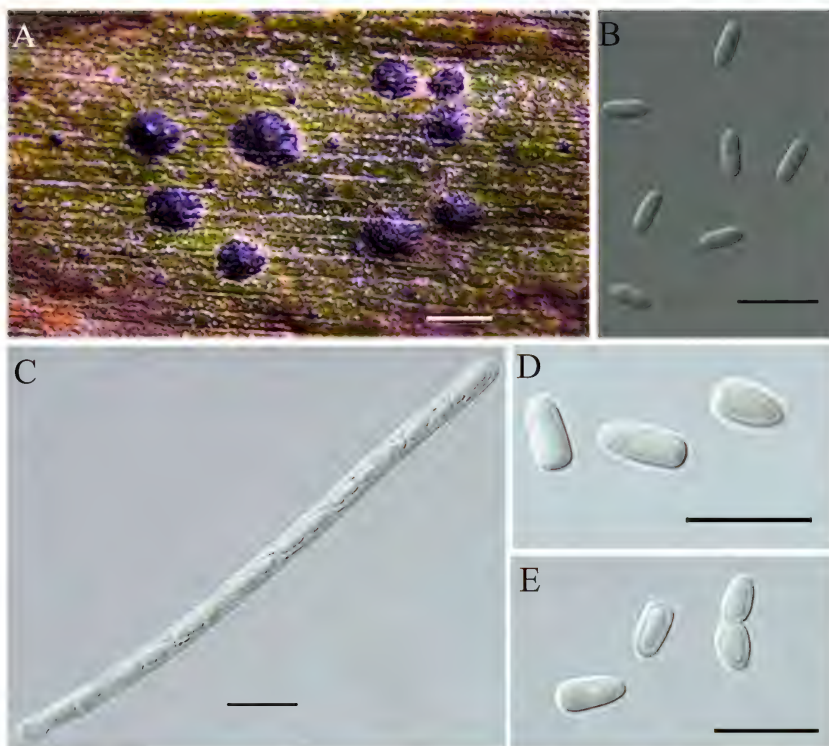


PLATE 1. *Strigula sinoaustralis*. A. Thallus with perithecia (holotype, HMAS-L 0137203); B. Macroconidia, with appendage at one or both ends (HMAS-L 0137204); C. Ascus, with eight uniseriate ascospores (holotype, HMAS-L 0137203); D, E. Ascospores, with distinct constriction at septum and breaking into parts outside the asci (holotype, HMAS-L 0137203). Scale bars: A = 300 μ m; B–E = 10 μ m.

The new species also resembles *S. nitidula* Mont., which is distinguished by a very thin (8–15 μ m) marginally effuse bright metallic green thallus usually bordered by a narrow black line (Lücking 2008).

Strigula microspora Lücking can be separated from *S. sinoaustralis* by its thallus with a white central part surrounded by crenulate or shortly lobulate margins, ascospores that do not fragment outside the asci, and larger macroconidia (8–10 $\times$ 2–2.5 μ m; Lücking 1991, 2008).

Strigula sinoaustralis cannot be confused with any species of the *S. smaragdula* group, which is characterized by broader asci and larger biseriate ascospores (Santesson 1952, Lücking 2008). Besides the morphological differences, the new species is also supported by the phylogenetic analysis (see p. 797).

Strigula antillarum (Fée) Müll. Arg., Bot. Jahrb. Syst. 6: 379 (1885). PL. 2A–E

THALLUS often medium green, 0.5–7.5 mm diam.

PERITHECIA exposed, immersed at the base, not sharply delimited from the thallus, not easily found in the specimens bearing pycnidia. ASCI 55–87.5 × 8–12 µm. ASCOSPORES 8 per ascus, biseriate, fusiform, with acute ends, 17.5–25 × 4–7.5 µm.

PYCNIDIA producing macroconidia aggregated. MACROCONIDIA bacillar, 1-septate, 12.5–20 × 3–5 µm.

CHEMISTRY. No substances detected by TLC.

SPECIMENS EXAMINED: CHINA. GUANGXI PROVINCE, Nanning, Long'an County, Longhu mountain natural reserve, 22°57'42"N 107°37'40"E, alt. 147 m, on living leaves, 1/XII/2015, S.H. Jiang GX201511110 (HMAS-L 0137209; GenBank KX216696), GX201511112 (HMAS-L 0137208; GenBank KX216697), GX201511114 (HMAS-L 0137207). HAINAN PROVINCE, Dongfang city, Nanlang village E'xian Ling, 19°00'18"N 109°04'09"E, alt. 160 m, on living leaves, 13/XII/2014, J.H. Wang & R.D. Liu HN2014376 (HMAS-L 0130571), HN2014354 (HMAS-L 0130622); 19°00'07"N 109°04'20"E, alt. 126 m, J.H. Wang & R.D. Liu HN2014393 (HMAS-L 0130573). YUNNAN PROVINCE, Xishuangbanna, Mengla County, tropical botanical garden of Chinese Academy of sciences, East area, 21°55'39"N 101°15'52"E, alt. 560 m, on living leaves, 18/XI/2015, X.L. Wei & S.H. Jiang XTBG2015051 (HMAS-L 0137211; GenBank KX216702); Lvshilin, 21°54'35"N 101°16'52"E, alt. 626 m, 18/XI/2015, X.L. Wei & S.H. Jiang XTBG2015119 (HMAS-L 0137210).

REMARKS: *Strigula antillarum* is characterized by its aggregated and confluent pycnidia in the center of the thallus. This species is anatomically similar to *S. smaragdula*, which is distinguished by its thallus-covered perithecia and dispersed pycnidia (Lücking 2008).

Strigula laureriformis Aptroot & Lücking, Biblioth. Lichenol. 97: 131 (2008).

PL. 2FG

THALLUS growing on bark, bright green to white green.

PYCNIDIA immersed-erumpent, wart-shaped, covered by thallus, with ostiole exposed outside. CONIDIA ellipsoid, 1-septate, hyaline, 7.5–10 × 2.5–4 µm, sometimes with appendage 15–25 µm long.

PERITHECIA not seen.

CHEMISTRY. No substances detected by TLC.

SPECIMENS EXAMINED: CHINA. XIZANG PROVINCE, Motuo County, alt. 1000 m, on living leaves, 1982, Y.G. Su 2532 (KUN 7537).

REMARKS: *Strigula laureriformis* (very typical of the genus; Aptroot et al. 2008) does not produce perithecia and is easily recognized by its corticolous habit, bright white green thallus, and simple 1-septate conidia.



PLATE 2. *Strigula antillarum*. A. Thallus with pycnidia (HMAS-L 0137209); B. Ascospores, fusiform, with acute ends, not breaking into parts outside the asci (HMAS-L 0137211); C, D. Asci, with eight biseriate ascospores (HMAS-L 0137211); E. Macroconidia (HMAS-L 0137209). *Strigula laureriformis* (KUN 7537). F. Thallus with pycnidia; G. Macroconidia. *Strigula prasina* (HMAS-L 0137213). H. Hypophyllous thallus; I. Asci, with irregularly arranged ascospores; J. Ascospores, fusiform-ellipsoid, 1-septate, with slight constriction at septum and not breaking into parts outside the asci. Scale bars: A = 200 μ m; B–E = 10 μ m, F = 800 μ m, G = 20 μ m, H = 300 μ m, I, J = 10 μ m.

Strigula prasina Müll. Arg., Flora 68: 343 (1885).

Pl. 2H–J

THALLUS hypophyllous, subcuticular, dispersed into patches, pale green.

PERITHECIA basally immersed, hemispherical, black. PARAPHYSES slightly branched. ASCI dense and compact, cylindrical, $52.5\text{--}70 \times 7.5\text{--}10 \mu\text{m}$. ASCOSPORES 8 per ascus, biseriate to irregularly arranged or occasionally uniseriate, fusiform-ellipsoid, 1-septate, with slight constriction at septum, $12\text{--}17 \times 4\text{--}5 \mu\text{m}$.

PYCNIDIA not seen.

CHEMISTRY. No substances detected by TLC.

SPECIMENS EXAMINED: CHINA. HAINAN PROVINCE, Ledong County, Jianfengling, on living leaves, 2009, J.C. Wei 046F1 (HMAS-L 0137213; GenBank KX216700), 046F2 (HMAS-L 0137212; GenBank KX216701).

REMARKS: *Strigula prasina* is typically a hypophyllous species with a thallus morphology rather similar to that of *S. concreta*. However, *S. concreta* is typically epiphyllous and has distinctly smaller ascospores, often broken into parts (Santesson 1952). The other hypophyllous species, *S. janeirensis* (Müll. Arg.) Lücking, mainly differs in its thinner thallus, larger perithecia, and much larger ascospores that easily fragment within the asci (Roux & Sérusiaux 1995, Lücking 2008).

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***Thelenella haradae* sp. nov., a saxicolous lichen from South Korea**

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ABSTRACT—A new species of saxicolous lichen is described, and named as *Thelenella haradae*. It occurs on exposed granite rocks of Mt. Daebang, Gyeongnam Prov., southern maritime Korea, and is similar to *T. brasiliensis* but differs in having wider ascospores (25–30 × 13–17 µm) and completely immersed ascomata.

KEY WORDS—East Asia, lichenized ascomycetes, biogeography, taxonomy

Introduction

During examination of pyrenocarpus lichens deposited in KoLRI herbarium (Suncheon National University), a new species of *Thelenella* from Korea was discovered.

The genus *Thelenella* Nyl. (*Thelenellaceae*) consists of 35 corticolous, foliicolous, muscicolous, or saxicolous taxa and is characterized by a crustose thallus with a chlorococcoid photobiont and immersed ascomata usually lacking an involucrellum (Mayrhofer 1987, Morse 2016). Most saxicolous *Thelenella* species are limited to maritime or oceanic climates and non-calcareous substrata (with one exception, *T. calcicola* C.A. Morse; Morse 2016). *Thelenella modesta* (Nyl.) Nyl. has recently been reported from Korea (Aptroot & Moon 2014). We now present a second record for the country, here described as *Thelenella haradae*.

Materials & methods

The specimen was collected from southern part of Korea in 2011 and deposited in the lichen herbarium of the Korean Lichen Research Institute, Suncheon, Korea

(KoLRI). The specimen was examined morphologically and sections were made by razor blade under an Olympus SZX-7 stereomicroscope. The thin hand-cut sections of thallus and ascomata were mounted in tap water and examined anatomically using an Olympus BX50 compound microscope with DIC optics connected to a Canon 5D digital camera. All measurements were made in water, but sections of ascomata or pycnidia were mounted in 10% aqueous solution of KOH for observing asci and ascospores in more detail. Ascospores were measured, and mean length and width values $\pm$ standard deviations and mean length/width ratio were calculated. The methodology for colour spot reaction tests and thin layer chromatography (TLC) followed Orange et al. (2010).

Taxonomy

Thelenella haradae J. Halda, Xin Y. Wang, J.A. Ryu & Hur, *sp. nov.*

PL. 1

MYCOBANK MB819398

Differs from *Thelenella brasiliensis* by its completely immersed ascomata and wider ascospores.

TYPE: South Korea, Gyeongnam Prov., Namhae County, Mt. Daebang, 34°51'00"N 127°59'26"E, alt. 87 m, on vertical face of an exposed granite rock, 29 April 2011, X.Y. Wang & J.A. Ryu 110271 (Holotype, KoLRI 13485).

ETYMOLOGY: The new species is named in honor of the well-known Japanese lichenologist Dr. H. Harada for his numerous contributions to our knowledge of pyrenocarpous lichens in East Asia.

THALLUS lichenized, epilithic, 2–5 mm diam., 400–600 μ m thick, gray to gray-green, smooth, $\pm$ glossy, verrucose to subsquamulose, thick, with prominent brown hypothallus. I–, KI–, composed mostly of oblong to spherical hyphae (ca. 5–8 μ m diam.) and partly of linear hyphae (especially in the basal parts). EPINECRAL LAYER well-developed, ca. 20 μ m thick, hyaline, composed of horizontally flattened hyphal remnants. CORTICAL LAYER 60–80 μ m thick, plectenchymatous, composed of hyphae 1.5–2 μ m diam., colourless. PHOTOBIONT LAYER continuous, 40–60 μ m thick. MEDULLA often present, white, 40–100 μ m high. PHOTOBIONT cells of trebouxoid green algae mostly 7–10 μ m diam., usually in vertically oriented clusters.

ASCOMATA perithecioid (pseudothecial), oval to pyriform, 350–400 μ m tall, 300–350 μ m broad, without involucrellum, completely immersed in thalline warts; the warts 0.5–1 mm across, usually with several perithecia, dome-shaped to hemispherical, the same colour as surrounding thallus, colourless to brown in upper parts around ostiola. OSTIOLUM not visible on

PLATE 1 (right). *Thelenella haradae* (holotype, KoLRI 13485). A, thalline warts with ascomata; B, subsquamulose thallus; C, cross section of thalline wart with ascoma; D, cross section of thallus with upper epinecral layer, cortical layer, and medulla; E, cross section of ascoma showing perithecial wall structure, hamathecium, asci with ascospores, and anastomosing paraphysoids



(mounted in water); F, cross section of ascoma with detail of ostiolum; G, H, young and mature ascospores; I, apical part of non-amyloid ascus with a young ascospore. Scale bars: A = 1000 μ m; B = 500 μ m; C, E, F = 100 μ m; D = 50 μ m; G–I = 10 μ m.

the thallus surface, pale brown in upper part. EXCIPULUM grayish to brownish in the upper parts, hyaline below, 25–30 μm thick in sides, 40–50 μm at base; subhymenium concave above, 20–30 μm thick at base. HYMENIUM 300–320 μm high and 250–280 μm wide. All hymenial elements I–, KI–. PERIPHYESES absent, PSEUDOPARAPHYESES septate, branched and anastomosing to form a network, even in thickness (1 μm). ASCI clavate, fissitunicate; endotunica universally thick, lacking prominent ocular chamber, cylindrical, 8-spored, 80–100 $\times$ 12–15 μm . Ascus apices thickened, non-amyloid, bilabiate (for terminology see Eriksson 1981).

ASCOSPORES uniseriate, rarely biseriate in the ascus, (22–)25–30(–33) $\times$ (10–)13–17(–17) μm [means = $27.2 \pm 2.5 \times 14.5 \pm 1.8 \mu\text{m}$ (n = 56); l/w ratio = 1.9], broadly ellipsoidal to oval, colourless at first, dark in maturity, muriform (with 7 transverse and 2–3 longitudinal septa, 25–35 visible cells), lacking perispore.

PYCNIDIA not seen in thalline warts and thallus sections.

ECOLOGY & DISTRIBUTION: *Thelenella haradae* is known only from the type locality, Mt. Daebang, on an island off the southern coastline of the Korean Peninsula, where it grows at low elevation on the vertical surface of exposed rock (granite), together with *Xanthoparmelia* sp.

COMMENTS—*Thelenella luridella* (Nyl.) H. Mayrhofer, known from Japan (Harada 1999), differs from *T. haradae* by its colourless ascospores, its black perithecial ostioles visible on thalline warts, the presence of pycnidia, and the lack of a hypothallus. *Thelenella melanospora* Etayo & H. Mayrhofer, described from Spain (Etayo & Mayrhofer 2003), differs by its smaller (18–22 $\times$ 9–12 μm) ascospores, its paler coloured hypothallus, and its different substratum (smooth bark of shrubs or small trees). *Thelenella fernandeziana* (Zahlbr.) H. Mayrhofer, described from Juan Fernandez Islands (Mayrhofer 1987), differs by its ascomata with dark pigmented ostiolum and its longer (30–45 $\times$ 15–22 μm) ascospores; and *T. brasiliensis* (Müll. Arg.) Vain., known from tropical South and Central America, Africa, and China, differs by its narrower ascospores (20–32 $\times$ 9–13 μm) and its emergent ascomata with a pale brown ostiolum (Mayrhofer 1987).

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Notes on rust fungi in China 2. Two species of *Coleosporium* on *Compositae*

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ABSTRACT—During investigations of rust fungi in northeast China, specimens of *Coleosporium* on various *Compositae* were collected. Based on comparative morphology and molecular phylogenetic analysis specimens on *Hieracium*, *Parasenecio*, and *Senecio* were identified as *C. neocacaliae*, and those on *Ligularia*, *Saussurea*, and *Synurus* as *C. saussureae*. *Hieracium umbellatum* is a new host for *C. neocacaliae*, and *Saussurea odontolepis* and *S. pulchella* are new hosts for *C. saussureae*. *Coleosporium synuricola* is treated as a synonym of *C. saussureae*.

KEY WORDS—*Pucciniomycetes*, rust flora, taxonomy, *Uredinales*

Introduction

The rust genus *Coleosporium* is characterized by 1-celled teliospores that are produced in a 1-layered crust under host epidermal cells (Cummins & Hiratsuka 2003). The teliospores germinate without dormancy and produce 4-celled internal basidia. About 240 species have been described (data from MycoBank) and most of the species have a heteroecious life cycle producing spermogonia and aecia on needles of pines and uredinia and telia on various angiosperms (Kaneko 1981). In China about 45 species have been reported (Miura 1928, Ito 1938, Tai 1979, Cao & Li 1999, You et al. 2010), but their taxonomy and host plants are not well circumscribed and life cycles are still not investigated.

During investigations of rust fungi in Jilin Province, northeast China, we collected rust specimens on species of *Compositae* (*Hieracium*, *Ligularia*,

Parasenecio, *Saussurea*, *Senecio*, *Synurus*). These rust fungi have been referred to the genus *Coleosporium*, but species identifications are sometimes confused because of morphological similarities among species reported on these host plants. After morphological examinations and phylogenetic analyses, specimens on *Hieracium*, *Parasenecio*, and *Senecio* were identified as *C. neocacaliae* and those on *Ligularia*, *Synurus*, and *Saussurea* were identified as *C. saussureae*. *Hieracium umbellatum* L. was recorded as a new host plant for *C. neocacaliae* and *Saussurea odontolepis* (Herder) Sch.-Bip. ex Herder and *S. pulchella* (Fisch.) Fisch. were recorded as new hosts for *C. saussureae*. The identity of *Coleosporium synuricola* Y. Xue & L.P. Shao, described on *Synurus deltoides* (Aiton) Nakai, was clarified, and it was shown to be identical to *C. saussureae*.

Materials & methods

Specimens

Specimens on *Compositae* were collected mostly in the vicinity of Changbai Mountain, Jilin Province, China, from 2013 to 2015. Some specimens deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU) were also examined. Their host plants were *Hieracium umbellatum*, *Ligularia fischeri* (Ledeb.) Turcz., *Parasenecio hastatus* (L.) H. Koyama ($\equiv$ *Cacalia hastata* L.), *Saussurea odontolepis*, *S. pulchella*, *S. umbrosa* Kom., *Senecio nemorensis* L., and *Synurus deltoides*. The specimens used for morphological observations and molecular analysis were deposited in HMJAU.

Morphological observations

Light microscopy (LM) was used to examine morphological characters including the size and shape of sori and spores. Spores or thin-sections of sori from specimens were mounted in a drop of lactophenol solution on glass slides for LM. Approximately 20 spores from each specimen were randomly chosen and the length, width, and wall thickness of spores were measured using Leica LAS X software attached to a Leica DM2000 microscope (Leica, Germany).

The surface features of spores were examined by scanning electron microscopy (SEM). For SEM, samples obtained from dry specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium using a Hitachi MC1000 Ion Sputter Coater and examined with a Hitachi SU8010 SEM operated at 5–7 kV.

DNA extraction and sequencing

The total genomic DNA was extracted from about 200 spores obtained from a single uredinium or telium. Spores were crushed between two sterilized glass slides and suspended in 30 μ l extraction buffer [10 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 50 mM KCl, 0.01% sodium dodecyl sulfate (SDS), 0.01% Proteinase K], and the suspensions were incubated at 37°C for 1 hour and 95°C for 10 min, followed by a 4°C soak (Suyama et al. 1996, Virtudazo et al. 2001). From the crude extract, 5–7 μ l

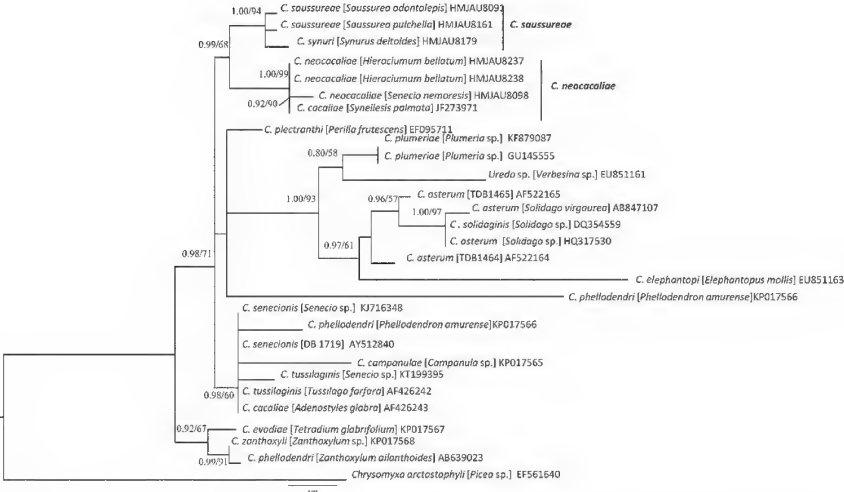


FIGURE 1. Phylogenetic tree of *Coleosporium*. Bayesian 0.5 majority-rule consensus tree based on the 28S rDNA gene. *Chrysomyxa arctostaphyli* was used as outgroup. Values on the branches indicate maximum likelihood bootstrap values / Bayesian posterior probabilities. Bootstrap values <75% and Bayesian posterior probabilities <0.75 are not shown on the branches.

samples were used directly for each polymerase chain reaction (PCR). The rDNA-28S region was amplified using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAG-3') and NL4 (5'-GGTCGCTGTTTCAAGACGG-3') (O'Donnell 1993). The PCR amplifications were performed in 50 µl of mixture containing 5 µl of template DNA, 200 µM of each primer, 25 µl of Premix Taq™ (TaKaRa Taq™ Version 2.0 plus dye) (TaKaRa, Tokyo, Japan), and 18 µl of ddH₂O. Cycling conditions for amplification consisted of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were separated on 1% agarose gels containing Nucleic Acid Stain (Beijing Dinggou Changsheng Biotechnology Co. Ltd.) and purified using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0. Purified PCR products were cloned in pEASY™-T1 Cloning Vector (Transgene Biotech, Beijing, China) and then transferred into Trans1-T1 phage, resistant chemically competent cell according to the manufacturer's instructions. The positive clones were sequenced by Sangon Biotech Co., Shanghai, China. All data sequenced in this experiment were deposited at GenBank (KX344988–344993).

Phylogenetic analysis

The sequence data were manually aligned using BioEdit ver. 7.0.9 (Hall 1999). To make molecular comparison, 23 sequences were retrieved from GenBank based on host species and genus (FIG. 1). Multiple alignments were performed using MEGA6. The final dataset contained sequences from 29 specimens with a length of 610 bp, which included 120 parsimony-informative characters.

Phylogenetic trees were constructed with a sequence of *Chrysomyxa arctostaphyli* Dietel as the outgroup. Topologies were constructed based on maximum likelihood (ML) analyses using RaxmlGUI1.5b1. Bayesian Markov chain Monte Carlo (MCMC) analyses were performed using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001). In ML and Bayesian analyses, the best-fit substitution models were estimated using Modeltest ver. 3.7 (Posada & Crandall 1998), and K81uf+I+G was selected as the best evolutionary model.

Results & discussion

Specimens on *Hieracium*, *Parasenecio*, and *Senecio*

Specimens on *H. umbellatum*, *P. hastatus*, and *S. nemorensis* were morphologically similar and their uredinial and telial characteristics were identical to the descriptions of *C. neocacaliae* (Kaneko 1981, Azbukina 2005). Molecular phylogenetic analysis of the rDNA 28S regions based on ML and Bayesian inference placed the specimens on *H. umbellatum* (HMJAU8237 and HMJAU8238) and *S. nemorensis* (HMJAU8098) in the same lineage (FIG. 1). GenBank data of a rust from Korea recorded as *C. cacaliae* (DC.) G.H. Oth on *Syneilesis palmata* (Thunb.) Maxim. [= *Cacalia thunbergii* Nakai] (JF273971)

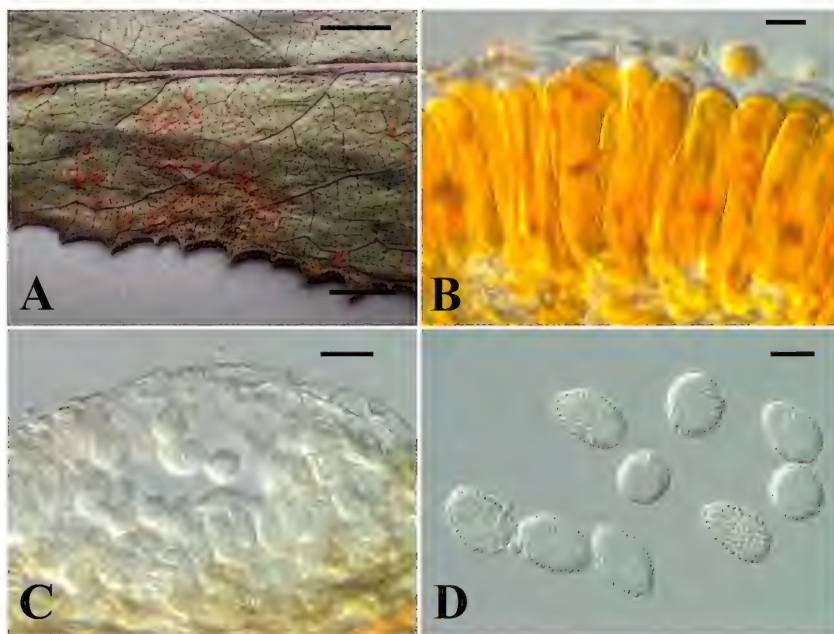


FIGURE 2. *Coleosporium neocacaliae* on *Hieracium umbellatum*. A. Telia on lower leaf surface. B. A vertical section of a telium. C. Vertical section of uredinium. D. Urediniospores. Scale bars: A = 1 cm; B–D = 20 μ m.

was also included in this lineage. However, this plant was reported as a host of *C. neocacaliae* (Kaneko 1981) and it is phylogenetically distinct from *C. cacaliae* from Europe (FIG. 1, AF426243). Based on these specimens a description of *C. neocacaliae* is provided.

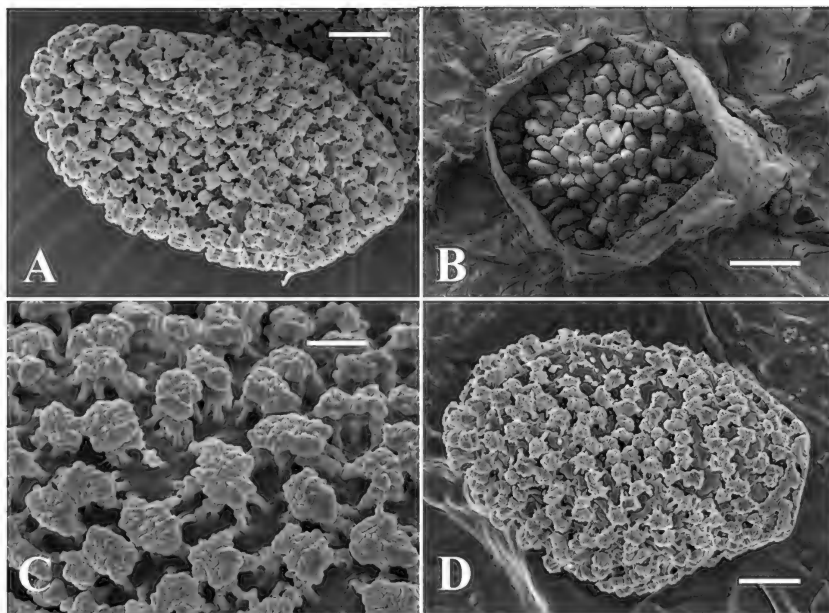


FIGURE 3. *Coleosporium neocacaliae*. A. Urediniospore on *Hieracium umbellatum*. B. Uredinium on *H. umbellatum*. C. Surface of urediniospore on *H. umbellatum*. D. Urediniospore on *Senecio nemorensis*. Scale bars: A, D = 5 μ m; B = 50 μ m; C = 2 μ m.

Coleosporium neocacaliae Saho, Trans. Mycol. Soc. Japan 7: 59, 1966. FIGS 2, 3

Uredinia hypophyllous, scattered, orange-yellow, pulverulent, globoid; urediniospores ellipsoid, broad-ellipsoid, 16.0–34.5 $\times$ 13.0–21.5 μ m (av. 24.5 $\times$ 16.5 μ m), walls uniformly thick 0.3–1.5 μ m (av. 1.0 μ m), verrucose with a smooth reticulum-like spot. Telia hypophyllous, scattered, rounded, orange-red; one-celled teliospores cylindrical, clavate or oblong-ellipsoid, 44–82 $\times$ 16–27 μ m excluding gelatinous apical layer, four-celled internal basidia 60–107 $\times$ 21–34 μ m, longitudinally or obliquely septate, mature teliospores or basidia arranged in a single layer, gelatinous apical layer 8–10 μ m thick, wall thin, hyaline.

SPECIMENS EXAMINED — CHINA, JILIN PROVINCE:

on *Hieracium umbellatum*: Changbai Mt., stage II, 29 July 2015 (HMJAU8237; GenBank KX344992); stage III, 23 September 2015 (HMJAU8238; GenBank KX344993);

on *Parasenecio hastatus*: Lushuihe, Baishan, stage II, 21 July 2002 (HMJAU8077, 8078, 8079); Changbai Mt., 29 July 2015, (HMJAU8263);

on *Senecio nemorensis*: Changbai Mt., stage 11, 23 July 2002 (HMJAU8098; GenBank KX344991).

Coleosporium neocacaliae has been reported only on species of *Cacalia* and *Senecio* from the Russian Far East, China, Korea, and Japan (Tai 1979, Kaneko 1981, Hiratsuka et al. 1992, Azbukina 2005), which makes *H. umbellatum* a new host plant for *C. neocacaliae*. This species differs in telial morphology from *C. cacaliae* and *C. parvisporum* S. Kaneko reported on species of *Cacalia* and *Senecio* (Kaneko 1981). The 4-celled basidia of *C. parvisporum* are smaller than those of *C. neocacaliae*. The 4-celled basidia of *C. cacaliae* have sterile cells at their base, but those of *C. neocacaliae* have no sterile cell. Our molecular analysis also confirms a phylogenetic difference between *C. neocacaliae* and *C. cacaliae* (FIG. 1).

Specimens on *Ligularia*, *Saussurea*, and *Synurus*

Specimens on *Ligularia fischeri*, *Saussurea odontolepis*, *S. pulchella*, *S. umbrosa*, and *Synurus deltoides* were morphologically similar and uredinial and/or telial characteristics were identical with the descriptions of *C. saussureae* (Kaneko 1981, Azbukina 2005). Our DNA sequence analyses also supported specimens on *Saussurea pulchella* (HMJAU8161), *S. umbrosa* (HMJAU8091) and *Synurus deltoides* (HMJAU8179) in the same lineage (FIG. 1). Therefore, these specimens were identified as *C. saussureae*. Rust on *Synurus deltoides* was reported as *C. synuricola* (Xue & Shao 1995, Azbukina 2005). However, we observed no morphological difference between specimens on *Ligularia* and *Saussurea* and so consider *C. synuricola* a synonym of *C. saussureae*. This synonymy is also supported by our molecular analysis. Based on these specimens a description is provided.

Coleosporium saussureae Thüm., Bull. Soc. Imp. Nat. Moscow 55: 212, 1880.

FIGS 4, 5

= *Coleosporium. synuricola* Y. Xue & L.P. Shao, Acta Mycol. Sinica 14: 248, 1995.

Uredinia hypophyllous, scattered, rounded, pulverulent, orange-yellow; urediniospores ellipsoid, oblong-ellipsoid or subglobose $21.5\text{--}32.5 \times 13.0\text{--}20.0 \mu\text{m}$ (av. $26.0 \times 16.0 \mu\text{m}$), verrucose, with a smooth reticulum-like spot, walls uniformly $0.5\text{--}2.0 \mu\text{m}$ (av. $1.0 \mu\text{m}$) thick. Telia hypophyllous, scattered, rounded, orange-red; one-celled teliospores obovoid to cylindrical, $48.5\text{--}88.5 \times 18.0\text{--}30.0 \mu\text{m}$ (av. $68.5 \times 23.5 \mu\text{m}$) excluding gelatinous apical layer, four-celled internal basidia $73.5\text{--}96.0 \times 18.0\text{--}27.0 \mu\text{m}$ (av. $81.5 \times 22.5 \mu\text{m}$), longitudinally or obliquely septate, mature teliospores or basidia arranged in a single layer, adhering among teliospores, gelatinous apical layer $9.5\text{--}32.5 \mu\text{m}$ thick, wall

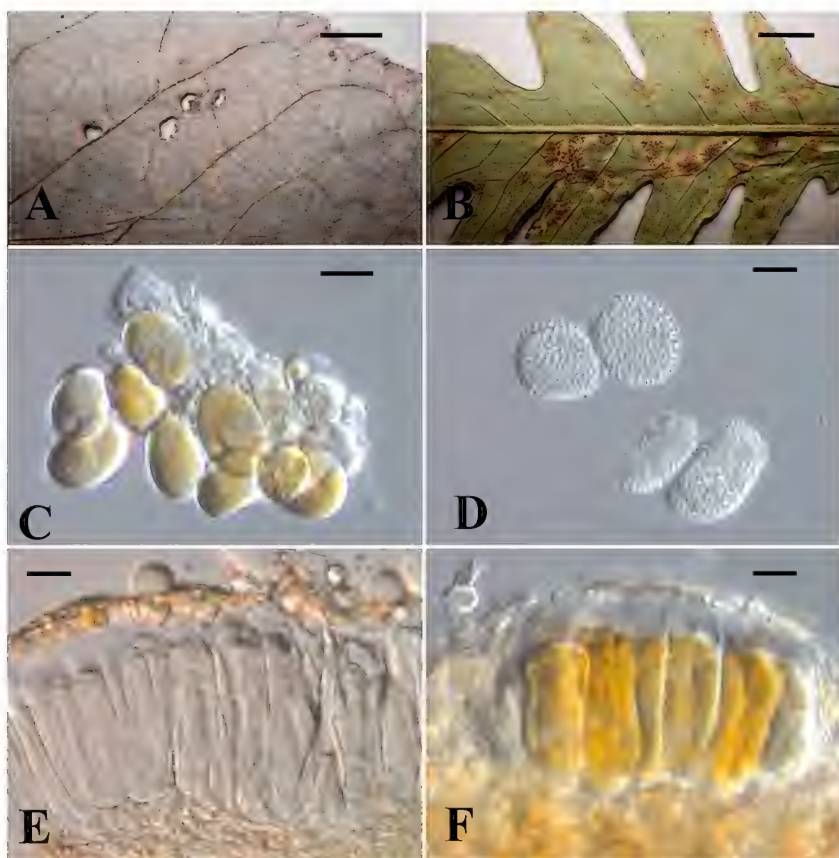


FIGURE 4. *Coleosporium saussureae*. A. Telia on lower leaf surface of *Synurus deltoides*. B. Telia on lower leaf surface of *Saussurea pulchella*. C. Basidiospores with smooth surface on *Synurus deltoides*. D. Urediniospores on *Saussurea umbrosa*. E. Teliospores on *Saussurea pulchella*. F. Teliospores on *Synurus deltoides*. Scale bars: A, B = 1 cm; C–F = 20 μ m.

thin, hyaline; basidiospores ellipsoid, $23.0\text{--}29.5 \times 15.0\text{--}18.5 \mu\text{m}$ (av. $25.5 \times 16.5 \mu\text{m}$), wall thin, hyaline.

SPECIMENS EXAMINED — CHINA, JILIN PROVINCE:

on *Ligularia fischeri*: Yanbian, stages II, III, July 2015 (HMJAU8186);

on *Saussurea odontolepis*: Changbai Mt., stages II, III, 26 August 2014 (HMJAU8088); 27 August 2014 (HMJAU8089);

on *S. pulchella*: Changbai Mt., stage II, 29 July 2015 (HMJAU8161: GenBank KX344988); stages II, III, 15 September 2013 (HMJAU8143); stage II, III, 16 September 2013 (HMJAU8163); stage II, III, 24 August 2014 (HMJAU8086); stage III, 24 August 2014 (HMJAU8087, 8090); stage III, 23 September 2015 (HMJAU8266, 8267); Yanbian,

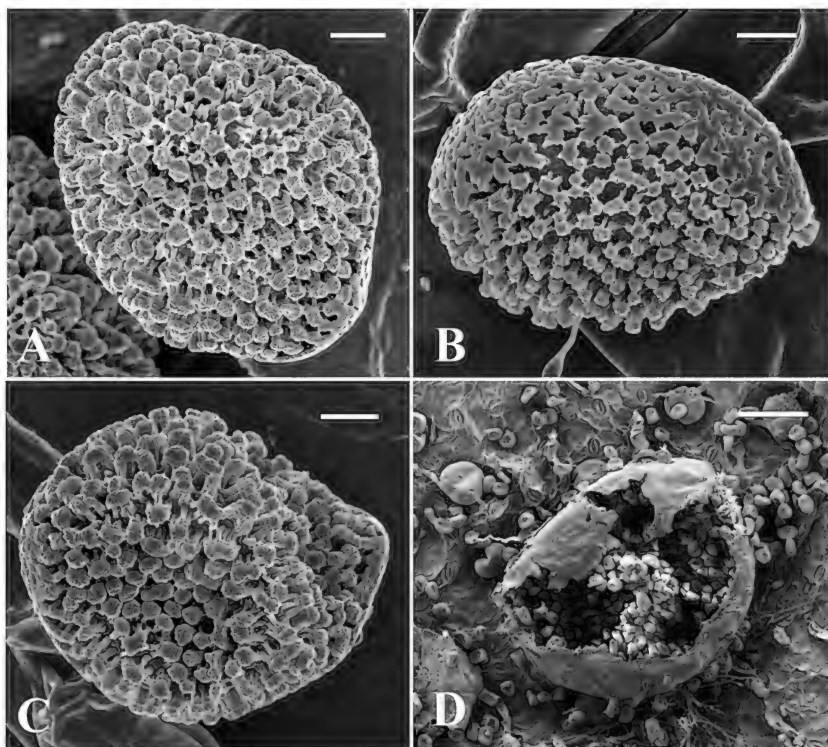


FIGURE 5. *Coleosporium saussureae*. A. Urediniospore on *Saussurea umbrosa*. B. Urediniospore on *S. odontolepis*. C. Urediniospore on *S. pulchella*. D. Uredinium on *S. pulchella*. Scale bars: A–C = 5 μ m; D = 50 μ m.

stage II, III, 30 July 2015 (HMJAU8165); stage II, III, 24 September 2015 (HMJAU8265); Yanji, stage III, 15 September 2013 (HMJAU8162, 8163, 8264); stage III, 17 September 2013 (HMJAU8146);

on *S. umbrosa*: Changchun, stage II, III, 11 September 2014 (HMJAU8091; GenBank KX344991);

on *Synurus deltoides*: Changbai Mt., stage III, 23 September 2015 (HMJAU8179; GenBank KX344989).

Coleosporium saussureae has been reported on *Ligularia* and *Saussurea* species and is known from the Russian Far East, China, Korea, Taiwan, and Japan (Kaneko 1981, Hiratsuka et al. 1992, Azbukina 2005). *Coleosporium pedunculatum* S. Kaneko was also described on species of *Saussurea*, but its teliospores have long sterile cells at their base, whereas *C. saussureae* has no sterile cell (Kaneko 1981). No sterile cells were observed in specimens on *Ligularia*, *Saussurea*, and *Synurus*. Therefore, we identified them as *C. saussureae*.

Ito (1938) and Tai (1979) listed *Saussurea odontolepis* and *S. pulchella* as host plants of *C. saussureae*. Later, Kaneko (1981) segregated *C. pedunculatum* from *C. saussureae* and treated them as host plants of *C. pedunculatum*, but specimens on these plants had no sterile cells on the teliospore bases and so were identified as *C. saussureae*. Therefore, these two plant species are new hosts for *C. saussureae* as revised by Kaneko (1981).

Geographical distribution of *C. saussureae* differs from that of *C. pedunculatum* due to the distributional differences of their aecial host species of *Pinus* (Kaneko 1981). *Coleosporium pedunculatum* is found mainly in warm regions, whereas *C. saussureae* occurs in alpine and mountainous regions, reflecting distribution of the aecial hosts. We collected specimens from Changbai Mt., an alpine (cold) region in China. Therefore, this identification of specimens is also supported by their distribution.

Coleosporium ligulariae Thüm. and *C. zhuangii* C.M. Tian & C.J. You have been recorded on species of *Ligularia* from China (You et al. 2010). These species were reported to differ slightly in size and urediniospore surface structure from *C. saussureae*, but they are morphologically similar to each other. Comparative morphological studies of these species are needed to clarify their relationships.

Coleosporium synuricola [= *Uredo synuri* Azbukina] was described based on a specimen collected on *Synurus deltoides* in Heilongjiang Province, China, which is geographically close to Jilin Province (Kaneko 1981, Xue & Shao 1995, Azbukina 2005). This species is morphologically similar to *C. saussureae* as reported by Kaneko (1981) and Xue & Shao (1995). We confirmed morphological and phylogenetic identity between them based on the specimen on *S. deltoides*. Therefore, we treat *C. synuricola* as a synonym of *C. saussureae*.

Acknowledgments

This work was financed by the National Natural Science Foundation of China (No. 31470646). We wish to thank Dr. E.H.C. McKenzie (Landcare Research, Auckland, New Zealand) and Dr. L.N. Vasilyeva (Institute of Biology and Soil Science, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia) for critical reading of the manuscript and suggestions. We express our thanks to Dr. B. Qu, Shenyang Agricultural University for identification of plant specimens.

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***Arthromoniliphora araucariae* gen. & sp. nov. from Brazilian pine**

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ABSTRACT—A new genus and species, *Arthromoniliphora araucariae*, is described and illustrated. This fungus is distinguished by differentiated hyaline conidiophores that are branched above the globose base and robustly cylindrical below, moniliform and subulate toward the apex. The conidia are thallic-arthric, cylindrical, bacilliform to cuneate, and hyaline and occur in unbranched chains formed by disarticulation of the moniliform, subulate conidiophore branches.

KEY WORDS—*Araucaria angustifolia*, asexual fungi, conidial fungi, taxonomy

Introduction

During a survey of hyphomycetes associated with plant litter in Floresta Nacional de São Francisco de Paula, Rio Grande do Sul State, Brazil, an interesting fungus was collected. This fungus shows remarkable differences from all previously described genera (Seifert et al. 2011) and is therefore described as a new genus and species.

Material & methods

Samples of litter of *Araucaria angustifolia* (Brazilian pine; Paraná pine) were placed in paper bags. In the laboratory the samples were placed in Petri dish moist chambers and stored in a 170 L polystyrene box with 200 mL sterile water plus 2 mL glycerol at 25°C for 30 days (Castañeda-Ruiz 2005). Mounts were prepared in PVL (polyvinyl

alcohol, lactic acid, and phenol). Measurements were made at a magnification of $\times 1000$. Micrographs were obtained with an Olympus BX51 microscope equipped with bright field and Nomarski interference optics. The type specimen is deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Arthromoniliphora S.S. Silva, Gusmão & R.F. Castañeda, **gen. nov.**

MYCOBANK MB 813427

Differs from *Arthrographis* and *Geotrichum* by its moniliform, closely fasciculate conidiophores and its conidia with a highly variable number of septa.

TYPE SPECIES: *Arthromoniliphora araucariae* S.S. Silva et al.

ETYMOLOGY: Greek *Arthro-*, meaning jointed (referring to thallic disarticulation) + *-monili-* Latin, meaning cylindrical but contracted at regular intervals + *-phora* referring to the conidiophores.

Conidial fungi. COLONIES on the natural substratum effuse, white. CONIDIOPHORES differentiated, single, densely fasciculate, branched, moniliform, septate, hyaline. CONIDIOGENOUS HYPHAE moniliform, subulate, holothallic, discrete, determinate. CONIDIAL SECESSION schizolytic. CONIDIA thallic-arthric, in unbranched chains, unpigmented, unicellular or multiseptate, cylindrical, doliiform or cuneate, forming by disarticulation of the terminal moniliform conidiogenous conidiophore branches.

Arthromoniliphora araucariae S.S. Silva, Gusmão & R.F. Castañeda, **sp. nov.**

MYCOBANK MB 813428

FIGS 1–3

Differs from all *Arthrographis* and *Geotrichum* spp. by its densely fasciculate conidiophores that are moniliform and cylindrical below and subulate above, and its cylindrical, unicellular to multiseptate conidia.

TYPE: Brazil, Rio Grande do Sul State, São Francisco de Paula, Floresta Nacional de São Francisco de Paula, 29°25'S 50°23'W, alt. 838 m, on decaying twig of *Araucaria angustifolia* (Bertol.) Kuntze (*Araucariaceae*), 10.III 2015, S.S. Silva (**Holotype**: HUEFS 216008).

ETYMOLOGY: Latin *araucariae*, meaning growth on an *Araucaria* species.

COLONIES on the natural substratum effuse, hairy, white. Mycelium superficial and immersed. Hyphae septate, branched, smooth, hyaline, 3–5 μm diam, sometimes forming a rudimentary globulose stroma. CONIDIOPHORES distinct, single, erect, compact fasciculate, branched just at one level near the base, moniliform, subulate toward apex of each branch, hyaline, multiseptate, 60–250 $\times$ 5–6 μm . CONIDIOGENOUS BRANCHES moniliform, subulate, holothallic, multiseptate, hyaline, discrete, determinate, 38–100 $\times$ 2.5–4 μm . CONIDIAL SECESSION schizolytic. CONIDIA thallic-arthric, in unbranched chains, hyaline, 0–36-septate, cylindrical, doliiform or cuneate, truncate at

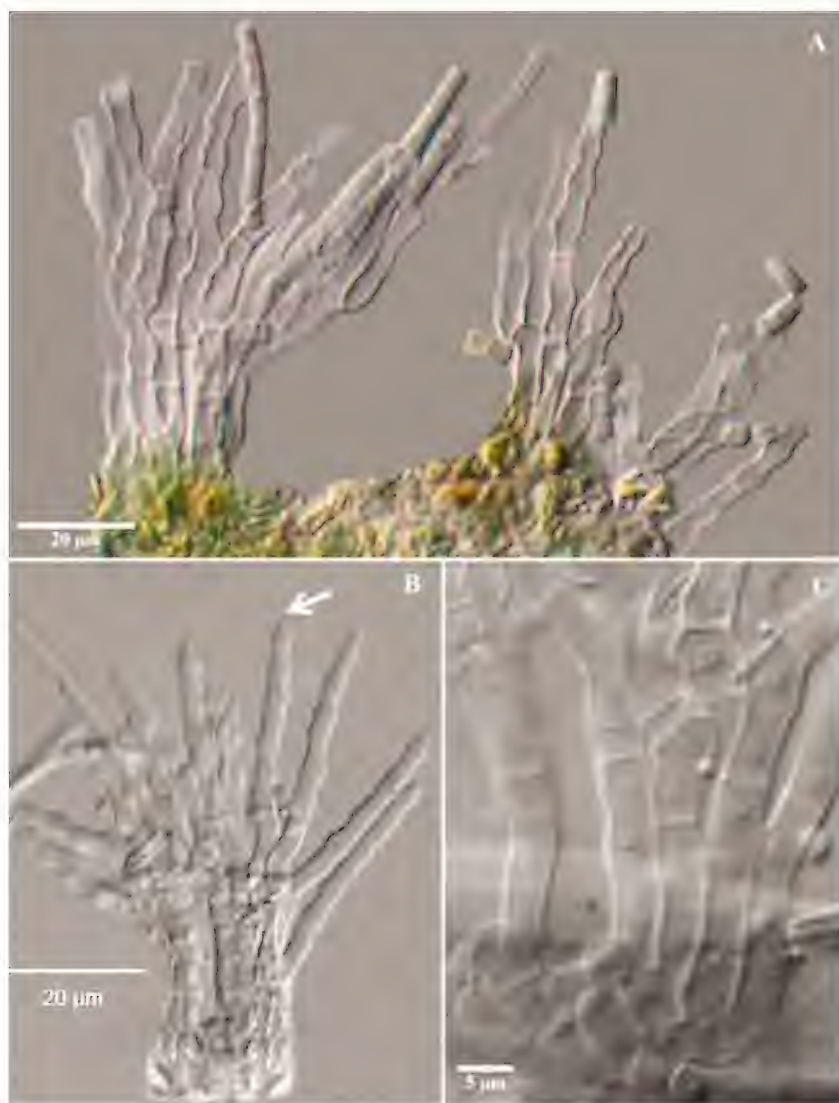


FIG. 1. *Arthromoniliphora araucariae* (ex holotype, HUEFS 216008). A, B. Conidiophores, conidiogenous branches, and conidia. C. Rudimentary stroma and basal part of conidiophores.

the ends, sometimes truncate at the base and obtuse at the apex, $3.5\text{--}92 \times 2.5\text{--}4 \mu\text{m}$ forming by disarticulation of the terminal, subuliform, moniliform conidiogenous branches of the conidiophores

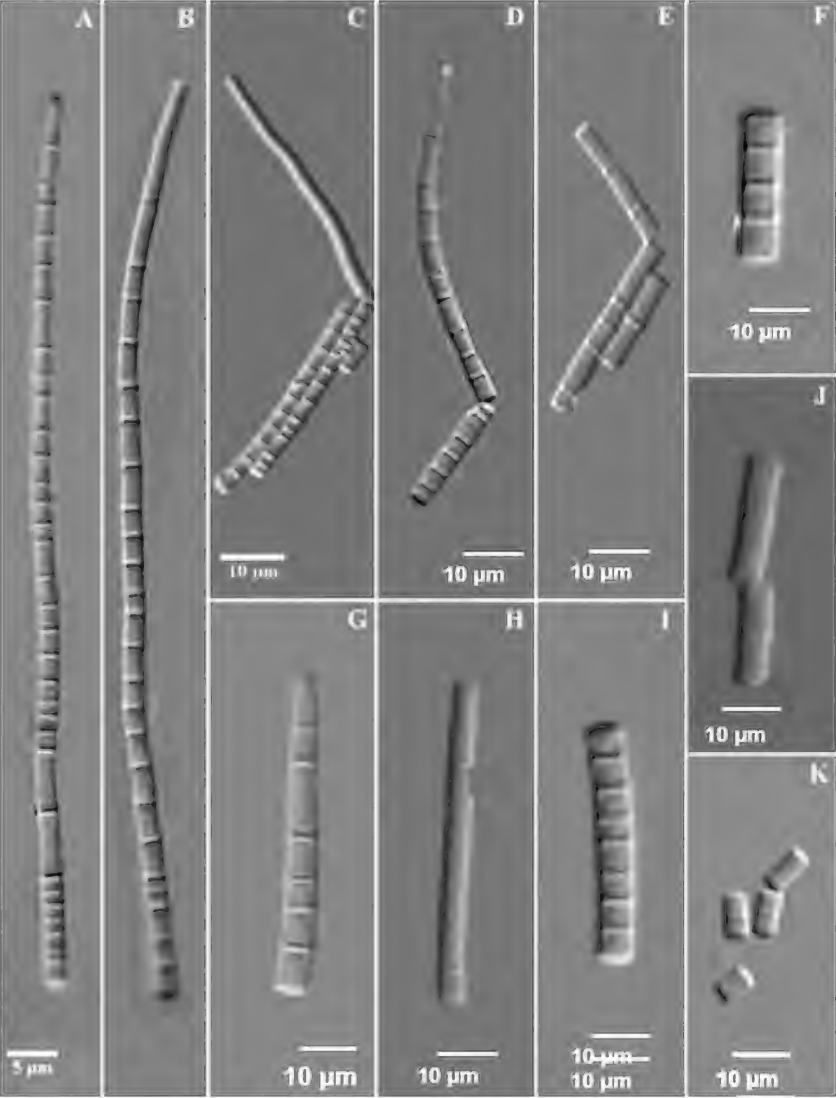


FIG. 2. *Arthromoniliphora araucariae* (ex holotype, HUEFS 216008).
Conidial chains and conidia.

COMMENTS—The genera *Arthrographis* G. Cochet ex Sigler & J.W. Carmich., *Geomyces* Traaen, and *Geotrichum* Link superficially resemble *Arthromoniliphora* in their hyaline thallic-arthric conidia produced by disarticulation of fertile hyphae. In *Arthrographis*, however, conidiophores are multi-branched, producing

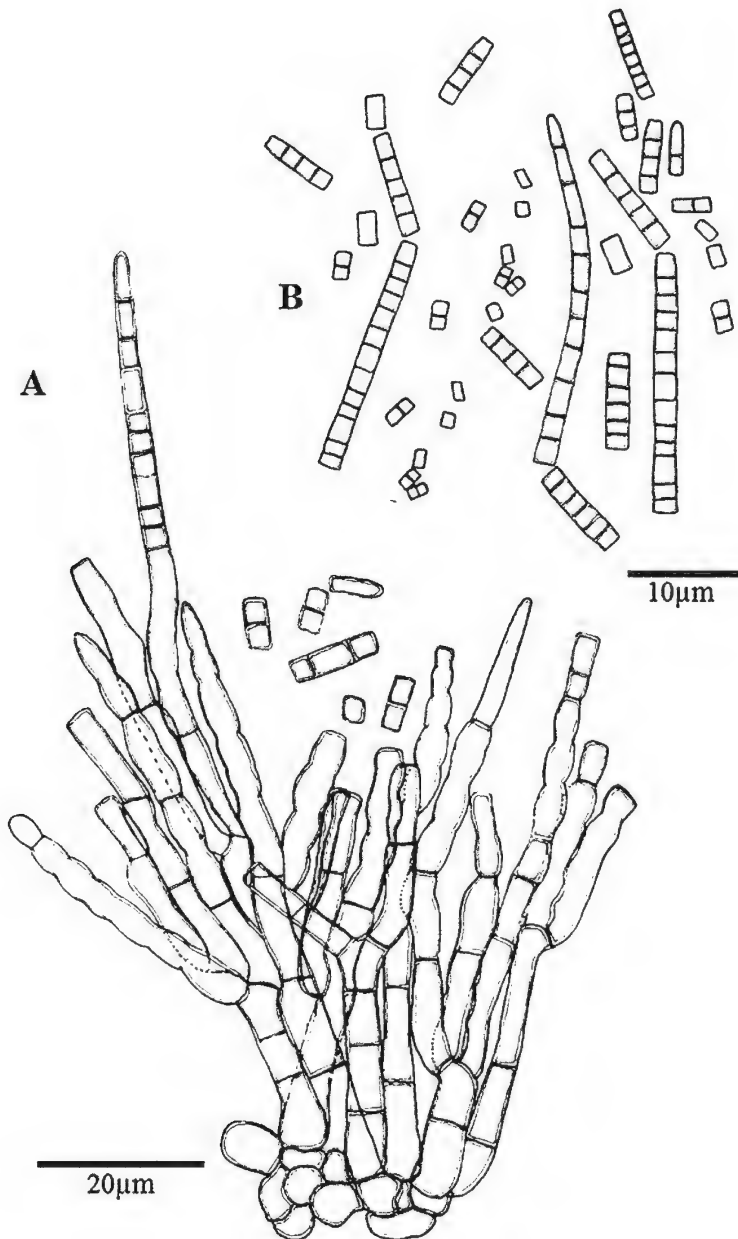


FIG. 3. *Arthromoniliphora araucariae* (ex holotype, HUEFS 216008).
A. Conidiophores, conidiogenous branches, and conidia. B. Conidia.

conidia that are globose, cylindrical, Y-shaped or irregular (usually discoid), rounded or subtruncate at the ends after disarticulation (Sigler & Carmichael 1976, Giraldo et al 2014). *Geomyces* has branched hyaline conidiophores, but the conidia have separating cells and the conidial secession is rhexolytic. *Geotrichum* is characterized by undifferentiated conidiophores, and after disarticulation of the fertile hyphae the conidia are unicellular, cylindrical, Y-shaped to irregular, sometimes forming chains, or slimy or yeast-like (Cole & Kendrick 1969, Sigler & Carmichael 1976, Hoog et al. 2011, Seifert et al. 2011). *Theadgonia* B. Sutton (Sutton, 1973) is somewhat similar to *Arthromoniliphora*, but its conidiogenous cells were described as polyblastic and sympodial and its conidia as blastocatenate, cylindrical, and 0–3-septate by Sutton (1973), while Seifert et al. (2011) described the conidial ontogeny as thallic-arthric. The *Theadgonia* conidiophores are branched and cylindrical but not moniliform and subulate as in *Arthromoniliphora*

Acknowledgments

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Morphological and phylogenetic clarification of *Peziza arvernensis*, *P. pseudovesiculosa*, *P. pseudosylvestris*, and *P. domiciliana*

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ABSTRACT—The concepts of the morphologically similar species *Peziza arvernensis*, *P. pseudovesiculosa*, *P. pseudosylvestris*, and *P. domiciliana* are revisited in light of new morphological and molecular data from freshly collected and herbarium specimens. Phylogenetic inference based on ITS nrDNA sequences suggests that *P. arvernensis*, *P. pseudovesiculosa*, and *P. pseudosylvestris* are monophyletic and that *P. domiciliana* represents an independent taxon.

KEY WORDS—Pezizales, taxonomy

Introduction

The distinction between *Peziza arvernensis* (subject of previous phylogenetic studies by Norman & Egger 1999 and Hansen et al. 2001, 2002), *P. pseudovesiculosa* [= *Aleuria amplissima* Boud.], *P. pseudosylvestris* [= *Galactinia pseudosylvestris*], and *P. domiciliana* is controversial because macro- and microscopic morphological characters seem insufficient to unambiguously define these taxa. Moreover, despite our several requests for samples of *P. pseudovesiculosa* and *P. pseudosylvestris* to institutional and private herbaria, we were able to obtain and examine only a few collections under these names.

Furthermore, *P. pseudovesiculosa* and *P. pseudosylvestris* are inadequately described (Gamundí 1975; Donadini 1977, 1978, 1979; Gamundí & Gaiotti 1998; Baiano et al. 2000; Moyne 2008). The two species seem to differ from

P. arvernensis primarily in the thickness of the layers of the excipulum, while all other characters overlap among the three species. Some authors (Hohmeyer 1986, Baiano et al. 2000) considered them to represent separate species, based on the thickness of the excipulum layers and a few other macroscopical and ecological features. The abundant observations reported by Donadini (1977, 1978, 1979, 1981) do not contribute significantly to a better delimitation of these taxa, because the characters he used are not decisive.

In the present work, we obtained new morphological and molecular data to clarify the phylogenetic position of these taxa, including: (1) Boudier's original material of *Aleuria amplissima* (PC 0084256, PC 0084257, PC 0084258), analyzed also by Donadini (1977) when he proposed the replacement name *P. pseudovesiculosa*; (2) original specimens described as *Galactinia pseudosylvestris* by Gamundi (1975); and (3) modern Italian specimens representing *P. domiciliana* Cooke.

Materials & methods

Material studied

The study was carried out on both fresh and dried samples (including holotypes) from the following herbaria—AMB, HMA, K, PC, MCVE, SIENA, TAAM, UPS—as well as from several private collections. The thirteen herbarium specimens selected for DNA extraction are listed in TABLE 1.

TABLE 1. Newly sequenced specimens of *Aleuria*, *Galactinia*, and *Peziza* used in phylogenetic analysis

SPECIES	COUNTRY	COLLECTOR & HERBARIUM NO.	ITS SEQUENCE
<i>A. amplissima</i>	France	E. Boudier PC 0084257	KC832902
	France	E. Boudier PC 0084258	KC832903
<i>G. pseudosylvestris</i>	Argentina	I. Gamundi (UPS) F-570657	KC832900
	Chile	I. Gamundi (UPS) F-570665	KC832899
<i>P. arvernensis</i>	Belgium	herb. D. Tanchaud DT 26051301	KP125489
	Italy	herb. F. Padovan FP 84	KC832897
	Italy	herb. A. Lantieri AL 0406055; K(M) 134792	KC832896
<i>P. domiciliana</i>	Italy	herb. G. Medardi AL 11030266; MCVE 16472	KC832906
	Italy	herb. G. Medardi GM 27058844	KC832907
	Italy	herb. F. Padovan FP 2014	KC832904
<i>P. pseudovesiculosa</i>	Italy	herb. G. Medardi GM 16100447	KC832901
	Italy	herb. G. Medardi GM 22099945	KC832898
	Italy	herb. G. Medardi GM 28100346	KC832905

SPECIMENS EXAMINED:

Peziza arvernensis: **BELGIUM**: VALLONIA, Fôret de Soignes Brabant Wallon, on the ground in a deciduous wood, 26.05.2013, leg. & det. D. Tanchaud (DT 26051301; GenBank KP125489). **DENMARK**: ZEALAND, Stenholdtsvang, Grib Skov, on sandy soil, 14.08.1965, leg. P.M. Petersen, det. H. Dissing (TAAM 187941). **ENGLAND**: **HEREFORDSHIRE**, Ross-on-Wye, on sandy soil, 19.06.1977, leg. & det. W.D. Graddon (K(M) 42798); **SURREY**, Sheepleas, West Horsley, on the ground in a deciduous wood with *Fagus*, 12.10.1981 (K(M) 50729); **WEST SUSSEX**, Madehurst, on the ground under deciduous trees, 2.07.1978, leg. D.A. Reid (K(M) 50718). **ESTONIA**: **TARTU CO.**, along the road to Tallinn, on the ground in *Fagus* litter, 13.05.2007, leg. L. Tedersoo, det. K. Hansen (TAAM 192338). **ITALY**: **ABRUZZO**, Prato di Campo secco, near woody residue, 10.10.1984 (HMA 3754); Teramo, Intermezzoli, Fonte Novello, Pietracamela, on the ground in a *Fagus* wood, 20.06.2004 (HMA 6216); **BASILICATA**, Potenza, Picerno, Monti li Foi, on the ground in a *Fagus* wood near fallen wood, 24.10.1998, leg. & det. G. Medardi (MCVE 14131); **CALABRIA**, Reggio Calabria, Molochio, on the ground in a mixed deciduous wood (mostly *Fagus*), 11.11.1988, leg. & det. F. Padovan (FP 159); Gambarie, near woody residue in a *Fagus* wood, 18.10.2003, leg. & det. G. Medardi (MCVE 16614); near woody residue in a *Fagus* wood, 6.06.2010, leg. & det. B. Recchia (BR 7386); **CAMPANIA**, Salerno, Camalunga, 11.06.2008, leg. G. Ruocco, det. L. Pecoraro & P. Leonardi (SIENA 6546); **EMILIA-ROMAGNA**, Reggio Emilia, Bergogno, Casina, on the ground in a *Fagus* wood, 28.04.1991, leg. & det. G. Simonini (MCVE 7282); **LAZIO**, Terni, Trevi del Lazio, 20.07.1986 (HMA 4128); Roma, Cervara di Roma, 30.07.1986 (HMA 4127, as *Peziza repanda*); **PIEMONTE**, Vercelli, Alagna Valsesia, near woody residue in a mixed deciduous wood, 25.08.1995, leg. & det. P.G. Jamoni (PGJ 931, as *Peziza pseudosylvestris*); **SICILY**, Catania, Maletto, Parco dell'Etna, near woody residue in a *Fagus* wood, 2.05.2004, leg. & det. A. Lantieri (AL); Messina, Cesarò, Parco dei Nebrodi, on the ground in a mixed deciduous wood, 4.06.2005, leg. & det. A. Lantieri (K(M) 134792, AL 0406055; GenBank KC832896); **TRENTINO-ALTO ADIGE**, Belluno, Monte Palmina, Canale d'Agordo, on the ground, 24.08.1996, leg. & det. E. Bizio (MCVE 10690); **TUSCANY**, Pisa, Calambrone, on sandy soil near *Cistus*, *Juniperus* and *Ammophila* in marine environment, 26.04.1996, leg. & det. G. Medardi (MCVE 11261); **VENETO**, Belluno, Campon, Bosco del Cansiglio, on the ground near *Fagus*, 25.07.1988, leg. & det. F. Padovan (FP 84; GenBank KC832897); Padola, on the ground, 21.08.2012, leg. & det. A. Testoni (GM 21081216); Tambre, Pian Osteria, Loc. Canaie, under deciduous, 7.07.2001, leg. G. Robich, det. E. Campo (MCVE 15980); Vicenza, Recoaro, Malga Chempelle, under deciduous, 5.06.2004, leg. & det. G. Medardi (MCVE 16797). **SCOTLAND**: **HIGHLAND**, Inner Hebrides, Isle of Skye, Armadale Castle, on sandy soil, 24.05.1982, leg. & det. R.W.G. Dennis (K(M) 50726); on sandy soil, 27.09.1983, leg. & det. R.W.G. Dennis (K(M) 50731); on sandy soil, 15.05.1984, leg. & det. R.W.G. Dennis (K(M) 50730); **PERTH AND KINROSS**, Birks of Aberfeldy, under deciduous, 2.08.2010, leg. J. & D. Bailey, det. B.M. Spooner (K(M) 166578). **SWEDEN**: **NÄRKE**, Lanna, on the ground, 12.07.1981, leg. & det. L. Södeberg (UPS F-640159).

Peziza domiciliana: **ITALY**: **ABRUZZO**, Aquila, Chiarino, near woody residue of *Fagus*, 24.05.2000 (HMA 12); Provvidenza, 2.10.2000 (HMA 519); **CALABRIA**, Reggio Calabria, Gambarie, near woody residue of *Fagus*, 7.11.1988, leg. & det. A. Para (AMB 000236, as *Peziza micropus*); on the ground under *Fagus*, 9.12.2000, leg. & det. G. Medardi (MCVE 15807); **LOMBARDIA**, Brescia, Ceto, on sandy soil under mixed deciduous trees, 27.05.1988, leg. & det. G. Medardi (GM 27058844; GenBank KC832907); **VENETO**, Belluno, near woody residue of *Fagus*, 15.04.1993, leg. & det. F. Padovan (FP 2014; GenBank KC832904), Padova, Monselice, on the ground under deciduous trees,

5.03.1997, leg. & det. G. Medardi (MCVE 12412); **SICILY**, Siracusa, R.N.O. Vendicari, on sandy soil in a mixed wood, 11.03.2002, leg. & det. G. Medardi (MCVE 16472, AL 11030266; GenBank KC832906).

Peziza pseudovesiculosa: **ITALY**: **EMILIA-ROMAGNA**, Reggio Emilia, Marola, on the ground under *Fagus*, 23.05.1997, leg. G. Baiano, det. D. Garofoli (MCVE 12816); **LOMBARDIA**, Brescia, Manerba del Garda, 22.09.1999, leg. & det. G. Medardi (GM 22099945; GenBank KC832898); Calvagese, on the ground under *Castanea*, 4.11.2000, leg. & det. G. Medardi (MCVE 15497); on the ground in a mixed deciduous wood (mostly *Carpinus* and *Alnus*), also near burnt residue, 28.10.2003, leg. & det. G. Medardi (GM 28100346; GenBank KC832905); Puegnago del Garda, under mixed deciduous trees, 16.10.2004, leg. & det. G. Medardi (GM 16100447; GenBank KC832901); **PIEMONTE**, Alessandria, Vignole Borbera, fraz. Variano Superiore, under deciduous trees, 22.04.2009, leg. & det. M. Carbone (MCVE 24022); **TUSCANY**, Siena, Riserva di Cornocchia, 26.05.2010, leg. H. Danielli, L. Pecoraro & E. Salerni, det. L. Pecoraro & E. Salerni (SIENA 6547); **VENETO**, Belluno, Falcade, Caviola, near *Fagus*, 15.08.1984, leg. & det. E. Bizio (EB 15088407).

Aleuria amplissima: **FRANCE**: **PARIS**, Île-de-France, Val d'Oise, Bois de Beauchamp et Forêt l'Isle-Adam, on sandy soil in the forest, s.d., leg. & det. E. Boudier (PC 0084256, holotype); on sandy soil in the forest, 04.1889, leg. & det. E. Boudier (PC 0084258; GenBank KC832903); on sandy soil in the forest, 04.1902, leg. & det. E. Boudier (PC 0084257; GenBank KC832902).

Galactinia pseudosylvestris: **ARGENTINA**: **TIERRA DEL FUEGO**, Ushuaia, on the ground in a deforested area, 21.01.1940, leg. & det. I. Gamundí (UPS F-570656 [containing 6 collections]; UPS F-570657 [containing 4 collections]; GenBank KC832900). **CHILE**: **TIERRA DEL FUEGO**, Magallanes y Antártica Chilena, Rio Rubens, on the ground in a *Nothofagus pumilio* forest, 11.01.1941, leg. & det. I. Gamundí (UPS F-570666 [containing 10 collections]); Canal Whiteside, Puerto Yartou, on the ground in a *Nothofagus pumilio* forest, 12.02.1941, leg. & det. I. Gamundí (UPS F-570665 [containing 4 collections]; GenBank KC832899).

Morphological studies

Measurements and descriptions of microscopic characters were made using material mounted in water, rehydrated when necessary with 5% KOH. Melzer's reagent and Cotton blue in lactic acid were also used. An Optika BK 1301 microscope with 40× or 100× (immersion oil) objectives was used to study and photograph the morphological features. Spore dimensions were calculated by measuring 50 mature spores.

DNA isolation, PCR, and sequencing techniques

Total DNA was extracted from dried specimens (TABLE 1) following a CTAB-based procedure. 600 µl of 2X CTAB-buffer was added to a small piece of ascoma and blended with the aid of a micropestle. It was then incubated at 65°C for 15 min; a volume of chloroform: isoamylalcohol (24:1) was added; and the mixture was gently shaken to emulsify both phases and centrifuged at 13,000 g.

One volume of isopropanol was added to the recovered supernatant, and the mixture was centrifuged again at 13,000 g for 15 min. The resulting pellet was washed in 70% ethanol, centrifuged for 2 min, dried, and finally eluted in 200 µl ddH₂O.

PCR amplifications each comprised a 45 µl working volume to which 1–2 µl of template DNA was added. Primer pairs ITS1F-ITS4, ITS1F-ITS2, or ITS3-ITS4 White

et al. (1990), and Gardes & Bruns (1993) were used to amplify nrDNA the ITS1 and ITS2 (or ITS1+2) regions. Amplification protocols were: 95°C initiation for 5 min; 35 cycles at 95°C for 45 s, 54°C for 30 s, and 72°C for 45 s; and a final step at 72°C for 10 min. PCR products were checked in 1% agarose gel prior to purification and sequencing with primers ITS1F or ITS4.

Chromatograms were visually checked in MEGA5 (Tamura et al. 2011) to detect ambiguous nucleotides and sequencing problems. Consensus sequences were placed in GenBank (TABLE 1). Newly generated sequences and their closest relatives in public databases were incorporated into the alignment produced by Hansen et al. (2002) stored in TreeBase (M1221), and only slight manual modifications were needed to accommodate insertions. Final analyses included 902 positions, of which 316 were parsimony-informative. A maximum parsimony (MP) search was performed in PAUP*4.0b10 (Swofford 2001) using standard conditions (2000 bootstrap replications, TBR swapping algorithm, 50 sequence additions per replicate, MULTREES not in effect). 50% majority-rule consensus tree bootstrap values were annotated in the best-scoring phylogram evaluated after a maximum-likelihood score analysis of all trees recovered. Finally, a Bayesian analysis was performed in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where the best-scoring model in MrModeltest 2.3 (Nylander 2004) was simulated. Six chains were employed with temperature set to 0.2, sampling every 100th generation until convergence parameters were met after 2,150,000 generations.

Results & discussion

The analyses were congruent with each other in the suggested topology (FIG. 1) and similar to the phylogenetic relationships already reported for this group (Hansen et al. 2002, Medardi et al. 2012).

Our results imply *P. pseudovesiculosa* [= *A. amplissima* Boud.] and *P. pseudosylvestris* as closely related to *P. arvernensis* within a monophyletic clade, sister to those formed by *P. varia* (Hedw.) Alb. & Schwein. and by *P. fimeti* (Fuckel) E.C. Hansen and related taxa. Within this monophyletic clade, the ambiguity of the taxonomic relationships among the different lineages could be subject to conflicting interpretations. We choose to consider them all as synonyms of *P. arvernensis* (the oldest available name) because of their insufficient genetic differences, the absence of diagnostic morphological features, and the similarly high intraspecific variability seen in the sister species *P. varia*. This synonymy can be summarised as:

Peziza arvernensis Roze & Boud., Bull. Soc. Bot. Fr. 26 (Suppl.): LXXVI. 1879.

= *Peziza pseudosylvestris* (Gamundi) Alessio, Micol. Ital. 4(2): 21. 1975.

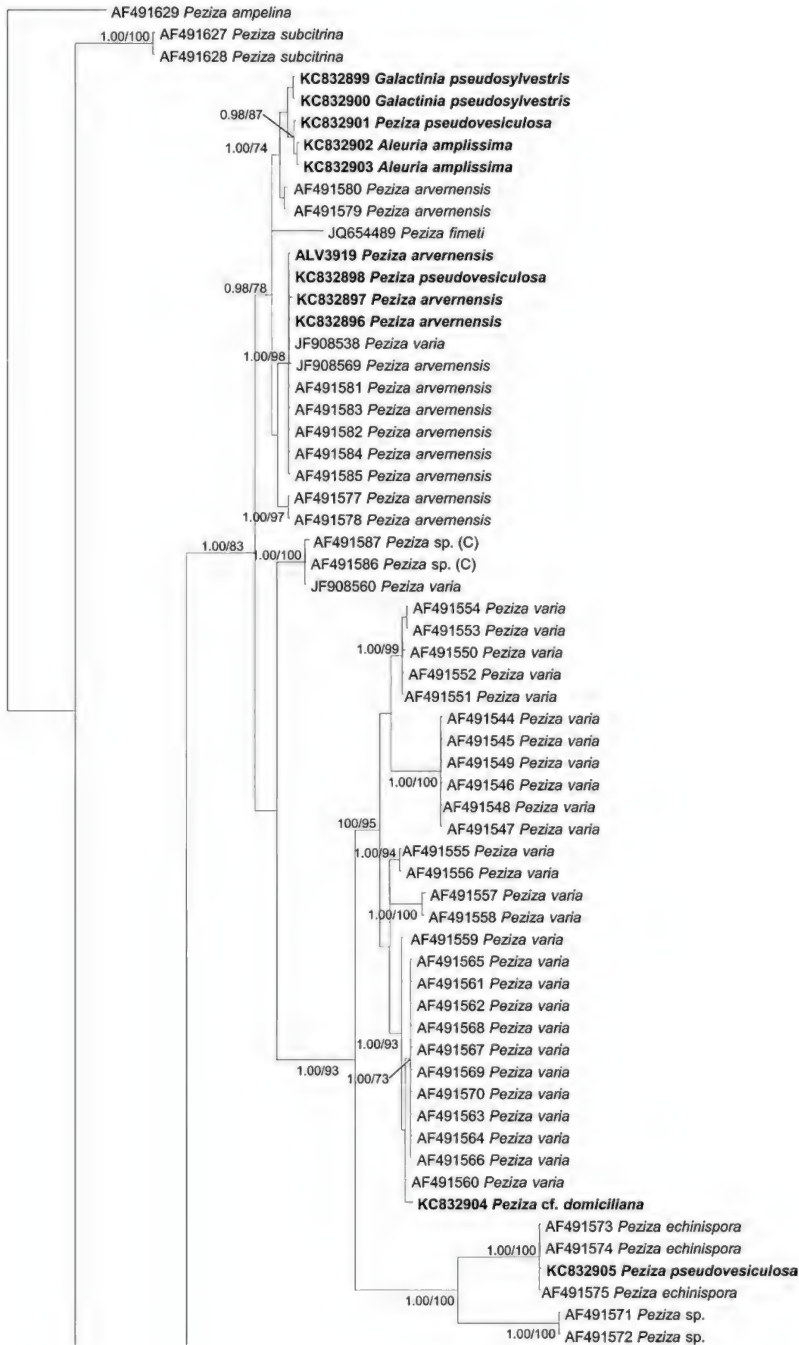
= *Galactinia pseudosylvestris* Gamundi, Fl. Cript. Tierra del Fuego 10(3): 37. 1975.

= *Peziza pseudovesiculosa* Donadini, Bull. Soc. Linn. Provence 30: 60. 1977.

= *Aleuria amplissima* Boud., Hist. Class. Discom. Eur.: 44. 1907, nom. illeg.
(non *A. amplissima* (Fr.) Pat. 1904).

= *Peziza amplissima* Sacc. & Traverso, Syll. Fung. 20: 309. 1911, nom. illeg.
(non *P. amplissima* Fr. 1849).

= *Galactinia amplissima* Svrček, Česká Mykol. 16: 110. 1962.



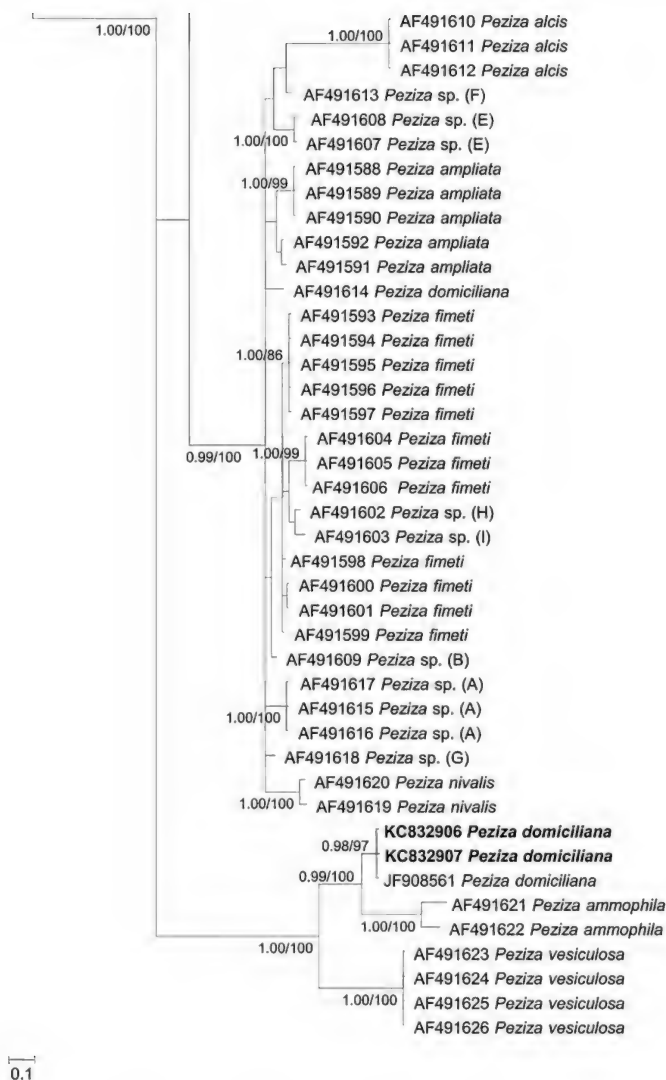


FIG. 1. Consensus ITS phylogram of *Peziza* s. str. obtained in MrBayes 3.1. Bayesian posterior probabilities and maximum parsimony bootstrap proportions are presented for nodes significantly supported by at least one of these analyses. Our new sequences are set in bold font.

TABLES 2–5 summarise morphological data for *P. arvernensis*, *P. pseudo-vesiculosa*, *P. pseudosylvestris*, and *P. domiciliana*, and make comparisons between data from the literature and from our study.

TABLE 2. *Peziza arvernensis*: comparison of cited and new morphological data

CHARACTER	ROZE & BOUDIER (1879)	CURRENT STUDY
APOTHECIUM	Regularly cup-shaped to undulate, fragile, rather thick, sessile	Often rather deep cup-shaped, tending to open in age, fragile, rather thick, sessile
HYMENIUM	Ochraceous-ferruginous with olivaceous tints	± Pale brown, brown-beige, sometimes pale tobacco, at times with reddish-violaceous or fawn tints
RECEPTACLE	Ochraceous-ferruginous with olivaceous tints, slightly furfuraceous. Base with white tomentum	Concolorous with the hymenium or greyish ochraceous-fawn with rust tints, tending to lighten when dried. Smooth to slightly furfuraceous
ASCOSPORES	16–18 × 8–9 µm, finely warted	16–19(–20) × 9–10(–11) µm, finely warted
ASCUS	(not reported)	270–280 × 15–16 µm
PARAPHYSES	Clavate above, septate, slightly coloured, with several oil droplets	Clavate, 6–8 µm above; subcylindrical, 3–4 µm below; septate
EXCIPULUM	(not reported)	SH: text. globulosa-angularis, cells 13–24 µm diam. ME: 3-layered (Upper & Lower layers: text. globulosa, cells 35–90 µm diam; Intermediate: text. intricata, hyphae 5–10 µm diam EE: text. intricata, hyphae 5–10 µm diam, mixed with globose cells 15–20 µm diam
HABITAT	Ground, also between mosses on one thatch	Ground, also sandy soil; marine to mountain environment, under deciduous trees (<i>Fagus</i> , <i>Quercus</i>), at times also near burnt residue or on woody remnants

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

The microscopical analyses of *Aleuria amplissima* revealed spores measuring 14–16(–18) × 8–9 µm, finely warted, with multiple small internal guttules and asci measuring 270–300 × 14–16 µm. We observed an ectal excipulum composed of septate hyphae 9–12 µm in diam. arranged in a textura intricata, more or less perpendicular to the hymenium and parallel to one another, instead of the textura globulosa-angularis reported by Donadini (1977, 1978, 1979) and Baiano et al. (2000). These characters are remarkably similar to those observed in several *P. arvernensis* collections: our observations of more or less finely warted spores of 16–19(–20) × 9–10(–11) µm and asci 270–280 × 15–16 µm agreed with those by Korf (1985). We also noticed a similar excipular structure and spore measurements in original material of *A. amplissima* (PC 0084256, PC 0084257, PC 0084258) and in *G. pseudosylvestris*.

Since the type material of *G. pseudosylvestris* in LPS was not available for study, other specimens collected and identified by Gamundí were analyzed

TABLE 3. *Peziza pseudovesiculosa*: comparison of cited and new morphological data

CHARACTER	DONADINI (1977)	CURRENT STUDY
APOTHECIUM	Cup-shaped, rarely opened, fragile, thick, sessile or nearly so	Cup-shaped, tending to open in age, fragile, $\pm$ thick, sessile or nearly so
HYMENIUM	Ochraceous to ochraceous-brown	$\pm$ Pale brown, brown, with some reddish tints
RECEPTACLE	Paler than the hymenium, furfuraceous	$\pm$ Concolorous with the hymenium, furfuraceous
ASCOSPORES	15–18 $\times$ 8–10 μ m, finely warted	16–19 $\times$ 9–10(–11) μ m, finely warted
ASCUS	240–270 $\times$ 14–16 μ m	270–280 $\times$ 15–16 μ m
PARAPHYSES	Cylindrical, 3–4 μ m below; clavate, 5–10 μ m above; septate	Subcylindrical, 3–4 μ m below; clavate, 6–8 μ m above; septate
EXCIPULUM	SH: text. globulosa-angularis, cells 7–15 $\times$ 15–20 μ m ME: 3-layered (Upper: text. globulosa-angularis, cells 15–90(–130) μ m diam.; Intermediate: text. intricata & porrecta, hyphae 6–12 μ m diam; Lower: text. globulosa, cells 30–80 μ m diam.) EE: text. globulosa, cells 15–25 μ m diam. with protruding hyphae 7–17 μ m diam.	SH: text. globulosa-angularis, cells 10–25 μ m diam. ME: 3-layered (Upper: text. globulosa, cells 30–80 μ m diam; Intermediate: text. intricata, hyphae 5–10 μ m diam; Lower: as first layer) EE: text. intricata, hyphae 5–10 μ m diam, mixed with globose cells 12–17 μ m diam.
HABITAT	Humus, woody remnants, burn residue	Humus, woody remnants, burn residue

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

(UPS-F570656, UPS-F570665, UPS-F570666, UPS-F570657). These samples showed spores measuring (15–)16–19(–19.5) $\times$ 8.5–11 μ m, with punctiform warts ca. 0.5 $\times$ 0.5 μ m and asci $\leq$ 280–320 $\times$ 12–14 μ m. Gamundí (1975) argued that *G. pseudosylvestris* could be distinguished from *P. arvernensis* by its thicker excipulum, especially the upper and lower layers of the medullar excipulum. Development of more or less wide cells in the excipulum and of moniliform or thread-like paraphyses seems, however, to be correlated with a more or less high level of humidity, as stated by Kullman (1995) and Hansen et al. (2002). Nonetheless, we observed an average thickness ca. 1500–1600 μ m in all rehydrated samples, in line with the measurements observed in “typical” *P. arvernensis*. We conclude that this species does not represent an independent lineage at the species level, but should be considered a synonym of *P. arvernensis*.

Most authors mentioned that *P. arvernensis* sensu stricto is typically associated with *Fagus sylvatica* L. (fruiting on soil, on litter, or near woody

TABLE 4. *Peziza pseudosylvestris*: comparison of cited and new morphological data

CHARACTER	GAMUNDI 1975; DONADINI 1978	CURRENT STUDY
APOTHECIUM	Cup-shaped, then more opened, subsessile to stalked	—
HYMENIUM	Pale ochraceous, pale hazel, ochraceous to olivaceous	—
RECEPTACLE	Concolorous to paler than the hymenium, furfuraceous	—
ASCOSPORES	14.1–18.2 (–19.2) × 7.2–9.6 µm, finely warted	(15–)16–19 (–19.5) × 8.5–11 µm, finely warted
ASCUS	240–355 × 9.6–14.4 µm	280–320 × 12–14 µm
PARAPHYSES	Thread-shaped, 2.4–4.3 µm large, septate, containing ochraceous granules	Subcylindrical, 3–4 µm below; clavate, 6–7 µm above; septate
EXCIPULUM	SH: text. globulosa, cells 8.4–15.6 µm diam. ME: 3-layered (Upper: text. globulosa, cells 50–120 µm diam; Intermediate: text. intricata, hyphae 4.8–9.6 µm diam; Lower: text. globulosa, cells 30–96 µm diam.) EE: text. intricata, hyphae 6–9.6 µm diam	SH: text. globulosa-angularis, cells ≤20 µm diam. ME: 3-layered (Upper: text. globulosa-angularis, cells up to 80 µm diam; Intermediate: text. intricata, hyphae 8–10 µm large; Lower: as first layer) EE: text. intricata, hyphae ≤5 µm diam, mixed with globose cells 15–20 µm diam.
HABITAT	Ground, near woody residuals of <i>Nothofagus</i>	—

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

remnants), but our observations show the habitat of *P. arvernensis* (both sensu stricto and sensu lato) to be quite variable: in addition to *Nothofagus*, some specimens have been collected under *Castanea* (MCVE 15497), *Carpinus*, *Alnus*, and other mixed deciduous trees in a floodplain environment (GM 28100346); *Quercus ilex* L. (AL 0406055); typical Mediterranean vegetation near *Cistus*, *Juniperus*, and *Ammophila* in a marine retro-dunal environment (MCVE 11261); and on ground with burn residues (MCVE 15497).

Peziza arvernensis sensu lato may also be confused with *P. domiciliana*, which has very similar features: a light umber-brown hymenial surface, paler (whitish-ochraceous to sub-concolorous) outer surface, spores measuring 15–17 (–20) × 7–9 (–10) µm that are both smooth and finely warted even in the same apothecium when ejected from the asci, a similar flesh architecture, and a substrate of soil, loam, or decaying wood. Sequences of two *P. domiciliana* collections (GM 27058844, AL 11030266) seem to match a sequence of another *P. domiciliana* stored in GenBank (JF908561; Garbellotto et al., unpubl.) that represents a lineage distinct from *P. arvernensis* (FIG. 1), thus supporting

TABLE 5. *Peziza domiciliana*: comparison of cited and new morphological data

CHARACTER	COOKE 1877	CURRENT STUDY
APOTHECIUM	Quite regularly cup-shaped, then cochleate and contorted, fragile	Cup-shaped, tending to open in age, fragile, rather thick, sessile or nearly so
HYMENIUM	Hues ranging from white to amethystine violet; sometimes rose-tinged	Amber-brown, honey coloured
RECEPTACLE	Concolorous	Paler than the hymenium, whitish-ochraceous
ASCOSPORES	11–13 × 6–7 µm, ornamentation not described	15–17(–20) × 7–9(–10) µm, smooth to finely warted
ASCUS	Not reported	270–300 × 13–15 µm
PARAPHYSES	Not reported	Subcylindrical 2–3 µm below, clavate 5–6 µm above, septate
EXCIPULUM	Not reported	SH: text. globulosa-angularis, cells ≤10–15 µm diam. ME: 3-layers: Upper & lower: text. globulosa, cells ≤50 µm diam; Intermediate: text. intricata, hyphae 5–10 µm diam EE: text. intricata, hyphae ≤5–10 µm diam
HABITAT	In a garden	Sandy or clay soil, loam, sawdust or other woody residue: also in cellars or caves

SH = Subhymenium; ME = Medullary excipulum; EE = Ectal excipulum

recognition of *P. domiciliana* as an independent species in accordance with Hansen et al. (2002). However, it should be noted that the sample of *P. domiciliana* sequenced by Hansen et al. (2002; AF491614) seems different from JF908561 and those sequenced in the present work and more closely related to the lineage formed by *Peziza ampliata* Pers., *P. fimeti*, and *Peziza alcis* Harmaja.

Hansen et al. (2002) noted that both *P. domiciliana* and *P. arvernensis* could be confused with *P. varia*. However, the spores in *P. varia* were observed by Medardi et al. (2012) as slightly smaller (14–16(–17.5) × 9–11(–12) µm), and the ornamentation in *P. varia*, very difficult to observe with a conventional light microscope in either surface or outline view, is readily visible in *P. domiciliana* and *P. arvernensis*. Nevertheless, one of our collections determined morphologically as *P. domiciliana* (FP 2014) fell instead in the clade of *P. varia*.

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***Podosporium bacilliforme* sp. nov. and a first record of *Phaeoblastophora peckii* from southern China**

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ABSTRACT—*Podosporium bacilliforme* is described and illustrated from specimens collected on dead branches in Guangxi Province. The fungus is characterized by black synnematal conidiomata and monotretic clavate conidiogenous cells producing solitary elongate-bacilliform phragmoconidia. *Phaeoblastophora peckii* is recorded for the first time in China.

KEY WORDS—conidial fungi, taxonomy

Introduction

Decaying wood in the tropical and subtropical forests of southern China supports a high level of fungal diversity, and several wood-inhabiting macrofungi and microfungi have been recently discovered there (Gao et al. 2015; Ma et al. 2011, 2014, 2015, 2016; Ren et al. 2012; Xia et al. 2014a, b, c; Zhang et al. 2008, 2011). During our ongoing surveys of saprobic fungi associated with woody debris in Guangxi and Hainan provinces, two interesting hyphomycetes were collected on dead branches. From morphological and developmental characteristics, we determined that one of these is an undescribed synnematosus species of *Podosporium*, while the other represents a first record of *Phaeoblastophora peckii* for China.

Material & methods

Samples were processed, examined and photographed following the methods described in Xia et al. (2014b). Adobe Photoshop 7.0 was used to process the photographs into compound images, with backgrounds modified for esthetic reasons. In view of our failure to obtain cultures of this species, no molecular data are presented here. The specimens are deposited in the Herbarium of Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

Podosporium bacilliforme J.Y. Wang, Yu M. Cai & X.G. Zhang, **sp. nov.**

FIG. 1

MYCOBANK MB 819468

Differs from all other *Podosporium* spp. by its larger bacilliform or elongated bacilliform conidia with many more eusepta.

TYPE: China, Guangxi Province: Tianyang county, Baiyao village, on dead branches of unidentified bamboo, 15 Nov. 2015, J.Y. Wang (**Holotype**, HSAUP H10119; **isotype**, HMAS 245637).

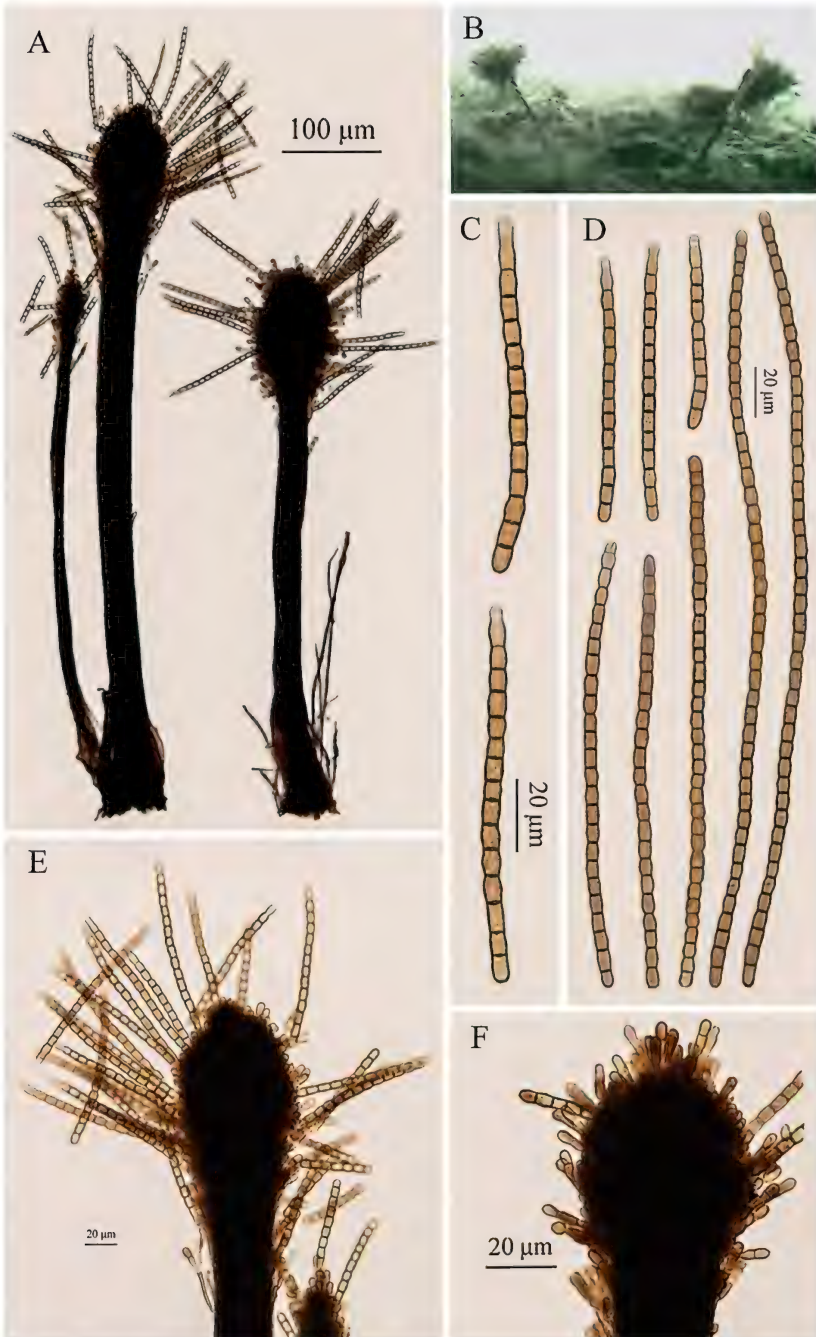
ETYMOLOGY: From the Latin *bacilliforme*, referring to the conidial shape.

COLONIES on the natural substratum effuse, brown. Mycelium mostly immersed, composed of septate, pale brown, flexuous branched hyphae. SYNNEMATA erect, cylindrical, black, solitary or in groups of 2–3, scattered, 470–720 µm long, 14–45 µm wide, up to 70 µm wide at the apical swollen region, expanded at the base. CONIDIOPHORES erect, arranged in synnemata, brown, smooth, septate, sometimes branched at the apex. CONIDIOGENOUS CELLS monotretic, clavate or cylindrical, integrated and discrete, terminal, determinate, pale brown, 5–8 × 3.5–4.9 µm. CONIDIA solitary, dry, smooth, bacilliform or elongate-bacilliform, base truncate, apex rounded, brown to dark brown, 77–310 × 4.5–6.0 µm, 9–43-euseptate, slightly constricted at the septa.

COMMENTS—*Podosporium* was erected by Schweinitz (1832) and typified by *P. rigidum* Schwein. The genus is characterized by subulate or cylindrical, darkly pigmented synnemata with monotretic, percurrent conidiogenous cells scattered along their length (Ellis 1971). More than 60 *Podosporium* species have been described (Index Fungorum 2016).

Podosporium bacilliforme bears some resemblance to *P. elongatum* J.L. Chen & Tzean, *P. etheldoidgeae* Crous et al., and *P. nilgirensis* (Subram.) M.B. Ellis in conidial shape. However, the conidia of *P. elongatum* (62–188 × 6–10 µm, 8–21-septate; Chen & Tzean 1993), *P. etheldoidgeae* (32–85 × 6–9 µm, 4–12-septate; Crous et al. 1995) and *P. nilgirensis* (32–50 × 7–9 µm, 4–6-septate;

FIG. 1. *Podosporium bacilliforme* (holotype, HSAUP H10119): A, B. Synnematos conidiomata, conidiogenous cells, and conidia. C, D. Conidia. E. Conidiogenous cells with conidia. F. Conidiogenous cells.



Ellis 1976) are much smaller and have many fewer septa than those in *P. bacilliforme*. Moreover, the terminal conidiogenous cells in *P. bacilliforme* clearly separate it from *P. etheldoidgeae*, which is not acropleurogenous.



FIG. 2. *Phaeoblastophora peckii* (HSAUP H7519): A. Conidiophores, conidiogenous cells, and conidia. B. Conidia. C. Conidiogenous cells and conidia.

Phaeoblastophora peckii (Sacc. & P. Syd.) Partr. & Morgan-Jones,
Mycotaxon 83: 342 (2002)

FIG. 2

COLONIES effuse, dark brown to black. MYCELIUM partly superficial, immersed, composed of branched, septate, brown to dark brown, smooth,

3–7 µm wide hyphae. CONIDIOPHORES single, distinct, arising as lateral branches, ascending, erect, straight or slightly flexuous, unbranched, cylindrical, inflated toward the extreme apex, brown to dark brown, slightly darker in the distal part, smooth, ≤280 µm long, 4.5–8 µm wide. CONIDIOGENOUS CELLS monoblastic or mostly polyblastic, integrated, terminal or intercalary, cylindrical, broadly clavate to capitate. CONIDIA acropleurogenous, simple or branched acropetal chains, dry, unicellular, smooth, sub-globose to ellipsoidal, dark brown, generally paler when young, usually narrowly truncate at one or both ends, unthickened at the ends, sub-globose 5–7 µm diam, ellipsoidal, 7–13 × 5.5–7 µm.

SPECIMEN EXAMINED: CHINA, HAINAN PROVINCE: Diaoluoshan, on dead branches of unidentified tree, 10 May 2014, X.Y. Li (HSAUP H7519).

COMMENTS—*Phaeoblastophora peckii* was originally described from America. Our specimen fits well with the description of the type material (Partridge & Morgan-Jones 2002), although the conidiophores of the Chinese specimen are slightly longer (280 µm vs 200 µm). Therefore, we believe they are the same species.

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Apiosordaria hamata sp. nov. from lake sediment in China

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ABSTRACT—A new species, *Apiosordaria hamata*, is described and illustrated from sediment collected from Donghu Lake, Wuhan, Hubei Province, China. *Apiosordaria hamata* is characterized by its cleistothecial ascomata with agglutinated hairs, a thin peridium, clavate asci, and two-celled, biserial, ovoid ascospores. However, it differs from other *Apiosordaria* species in having hooked spines on the upper cell of the ascospores and lacking germ pores. Combined ITS and LSU sequence analyses using maximum likelihood and Bayesian analysis nest *A. hamata* in the *Apiosordaria* clade. Both morphological and molecular analyses support this fungus as a new species of *Apiosordaria*. A key to *Apiosordaria* species is provided.

KEY WORDS—phylogenetic analyses, soil fungi, *Lasiosphaeriaceae*, *Sordariales*, taxonomy

Introduction

Arx & Gams (1967) introduced the genus *Apiosordaria* Arx & W. Gams (*Lasiosphaeriaceae*, *Sordariales*) based on the type species *Pleurancea verruculosa* C.N. Jensen [= *A. verruculosa*]. Krug et al. (1983) synonymised *Echinopodospora* and *Lacunospore* under *Apiosordaria* based on morphological similarities. In *Apiosordaria* ascospores are usually two-celled, composed of a dark upper cell and a lower hyaline cell; the upper cell is ellipsoidal to subglobose, with walls ornamented with numerous broad or narrow pits; the lower cell is triangular to cylindrical and possessing a minute hyaline terminal appendage (Arx & Gams 1967). In addition, the presence of spines, warts, or pits on the upper ascospore cell is used as a principal character that differentiates this genus from related

genera in the *Lasiosphaeriaceae* that also produce two-celled ascospores (e.g., *Cercophora*, *Podospora*, *Triangularia*, *Zopfella*; Garcia et al. 2003).

Apiosordaria currently comprises 23 species with a worldwide distribution in garden soils (Stchigel et al. 2000), field soils (Jensen 1912), grassland soils (Warcup 1951a), and forest soils (Warcup 1951b). The genus is also found in dung (Krug et al. 1983) and decaying wood (Badura & Badurowa 1964). Some species have also been found associated with plant material (Rostrup et al. 1916) and the insect *Plecia nearctica* (Kish et al. 1974).

During a study of the fungal diversity of wetlands in China, an interesting *Apiosordaria* species was isolated. Morphological characteristics distinguishing this taxon from all other species in the genus (Krug et al. 1983, Guarro & Cano 1988, Stchigel et al. 2000) are here supported by phylogenetic analyses. In this paper, the new species is introduced, described, and illustrated, and compared with the most similar species.

Materials & methods

Collection, isolation, and morphological examination

Soil samples were collected from Donghu Lake (30°33'N 114°23'E) in the northeastern outskirts of Wuchang, Wuhan City, Hubei, China. Donghu Lake, situated on the alluvial plain in the middle basin of the Changjiang (Yangtze) River, has a total surface area of 32 km² and lies 21.4 m (20.8–21.7 m) above sea level. The lake is usually 3–4 m deep with a maximum of 4.5 m, and the flat muddy lake bottom is sparsely covered with hydrophytes. The area is dominated by a damp subtropical monsoon climate, with a temperature range of 12.9–21.5 °C and annual precipitation of ca. 1040 mm. Sediment samples were collected along the bank of the lake at depths of 0–40 cm. The samples were sealed in plastic bags, placed in a 4 °C car refrigerator, and transported to the laboratory for processing.

A 10 g sediment subsample was homogenized, serially diluted in sterile distilled water, shaken, and 200 µl plated onto ¼ strength potato dextrose agar (PDA) containing 100 mg l⁻¹ streptomycin and 100 mg l⁻¹ chloromycetin. The plates were kept at 25 °C for 1 week, after which fungi with different colony morphologies were selected and grown on new PDA plates with antibiotics, on oatmeal agar (OA), potato carrot agar (PCA, potato 20 g, carrot 20 g, agar 20 g) and malt extract agar (MEA). Isolates were identified based on morphological characteristics after 10 days incubation at 25 °C.

Microscopic characteristics of the new species of *Apiosordaria* were observed with a Nikon Ellipse 80i light microscope equipped with differential interference contrast (DIC) optics and a FEI Quanta 200 scanning electron microscope (SEM). Ex-type and isotype cultures are deposited in the China General Microbiological Culture Collection Center (CGMCC), Beijing, China.

DNA sequencing & phylogenetic analysis

After 10 days growth on PDA, ca. 0.2 g of mycelium was collected with a sterile scalpel and placed into a 1.5 ml centrifuge tube. Genomic DNA was extracted with

TABLE 1. Sources and sequences used in the phylogenetic analysis.

TAXON	COLLECTION NO.*	ORIGIN	GENBANK ACCESSION NO.	
			ITS	LSU
<i>Apiosordaria backusii</i> [= <i>Triangularia backusii</i>]	CBS 106.77	Sandy soil, Japan	GQ922520	—
<i>A. hamata</i>	CGMCC 3.15230	Wetland soil, China	KP878306	KP878304
<i>A. hamata</i>	CGMCC 3.15231	Wetland soil, China	KP878307	KP878305
<i>A. longicaudata</i>	CBS390.84	Soil, Japan	—	FR692340
<i>A. nigeriensis</i>	FMR 6363	Garden soil, Nigeria	AJ458184	—
<i>A. tetraspora</i>	CBS 363.84	Soil, Thailand	—	FR692341
<i>A. verruculosa</i>	F-152365	Ethanol-pasteurized soil, Spain	—	AY346258
<i>A. verruculosa</i> var. <i>maritima</i>	CBS 550.66	Ethanol-pasteurized soil, Spain	—	FR692345
<i>Apodus decidiuus</i>	CBS 506.70	—	AY681199	AY681165
<i>A. oryzae</i>	CBS 376.74	—	AY681200	AY681166
<i>Bombardia bombardia</i>	AFTOL 967	—	—	DQ470970
<i>Bombardioidea anartia</i>	HHB99-1	Moose dung	—	AY346264
<i>Cercophora areolata</i>	UAMH7495	—	AY587911	AY587936
<i>C. sparsa</i>	JF00229	—	—	AY587937
<i>C. sulphurella</i>	SMH2531	—	AY587913	AY587938
<i>Cladorrhinum</i> sp.	MJ-2014	—	KJ572182	—
<i>Immersiella caudata</i>	SMH3298	—	—	AY436407
<i>I. immersa</i>	SMH2589	—	—	AY436408
<i>Jugulospora rotula</i>	ATCC38359	Branch, Panama	—	AY346287
<i>Lasiosphaeria glabrata</i>	TL4529	—	AY587914	AY436410
<i>L. lanuginosa</i>	SMH3277	—	AY587920	AY587943
<i>L. ovina</i>	CBS 958.72	Decaying wood, Germany	AY587925	AY587946
<i>L. rugulosa</i>	SMH4438	—	AY587932	AY587952
<i>Podospora cupiformis</i>	CBS 246.71	<i>Kobus defassa</i> dung	AY999125	AY999102
<i>P. curvicolla</i>	IFO 8548	Rabbit dung	AY999122	AY999099
<i>P. intestinacea</i>	CBS 113106	Horse dung	AY999121	AY999104
<i>Schizothecium aloides</i>	CBS 879.72	Soil, Netherlands	AY999120	AY999097
<i>S. fimbriatum</i>	CBS 144.54	Horse dung	AY999115	AY999092
<i>S. glutinans</i>	CBS 134.83	<i>Arctostaphylos uva-ursi</i> , Switzerland	AY999116	AY999093
<i>Strattonia carbonaria</i>	ATCC 34567	Burned soil, Japan	—	AY346302
<i>Triangularia mangelotii</i>	ATCC 38847	Soil, Japan	—	AY346303
<i>T. tanzaniensis</i>	TRTC51981	—	—	AY780081
<i>Xylaria hypoxylon</i>	AFTOL-ID 51	Downed rotting wood	DQ491487	AY544648
<i>Zopfiella tabulata</i>	CBS 230.78	Porcupine dung, Canada	AY999132	AY999105
<i>Zygopleurage zygospora</i>	SMH4219	Dung of cow, USA	—	AY346306

* ATCC, American Type Culture Collection, USA; AFTOL, Assembling the Fungal Tree of Life, USA; CBS, Centraalbureau voor Schimmelcultures, Netherlands; CGMCC, China General Microbiological Culture Collection Center, China; HHB, Field Museum of Natural History, Chicago, Illinois; IFO, Institute for Fermentation, Osaka; SMH, Sabine M. Huhndorf; TL, Thomas Læssøe; UAMH, University of Alberta Microfungus Collection and Herbarium.

a simple and rapid ‘thermolysis’ method (Zhang et al. 2010). Partial 28S rDNA and complete ITS+5.8S rDNA were amplified using fungal specific primers LROR/LR7 (Vilgalys & Hester 1990) and ITS5/ITS4 (White et al. 1990), respectively.

All PCR amplifications were carried out using a Biometra T-professional thermocycler. The reaction mixture included 5 µl of 10× Takara Bio PCR buffer, 2 µl of 25 mM MgCl₂ (1 µM final concentration), 4 µl of 2.5 mM deoxynucleotide triphosphate mix (final concentration, 250 µM each; Takara Bio, Japan), 2 µl of 10 µM primers each (final concentration, 0.25 µM; Sangon Biotech, China), 1 µl Sigma deionized formamide, and 0.4 µl Taq polymerase. The amplification was carried out as follows: 95 °C for 3 min; followed by 35 cycles at 95 °C for 1 min, 54 °C for 45 s, and 72 °C for 90 s; and a final extension for 10 min at 72 °C. After the PCR products were separated in 1% (w/v) agarose gels, they were stained with 0.4% GoldView nucleic acid gel stain (SBS Genetech) and viewed and photographed on a Gel imaging system (Bio-Rad).

The internal transcribed spacer (ITS) region and large-subunit rRNA (LSU) gene data sets were constructed with sequences of *Apiosordaria hamata* (isolates CGMCC 3.15230, CGMCC 3.15231) and reference sequences from related taxa retrieved from GenBank (TABLE 1). All sequences were aligned with ClustalX v1.83 (Thompson et al. 1997), and the alignment was manually adjusted to allow maximum alignment and minimize gaps. Maximum likelihood (ML) and Bayesian analysis were used to estimate phylogenetic relationships of *A. hamata* with its related taxa, with *Xylaria hypoxylon* (L.) Grev. as the outgroup taxon.

Maximum Likelihood analysis was conducted in RaxmlGUI v1.3 (Silvestro & Michalak 2012) with the GTRGAMMAI model, 1000 bootstrap replicates. While Bayesian analysis was performed in a likelihood framework as implemented by MrBayes v3.0b4 software package to reconstruct phylogenetic trees (Huelsenbeck & Ronquist 2001), the best-fit evolutionary model was determined by MrModeltest v2.3 (Posada & Crandall 1998, Nylander 2004) through comparing different nested DNA substitution models in a hierarchical hypothesis-testing framework. The best-fit model determined by MrModeltest was the “GTR+I+G”, lsetnst = 6, rates = invgamma; prset statefreqpr = dirichlet (1, 1, 1, 1) for Bayesian analyses. Multiple Bayesian searches using Metropolis-coupled Markov chain Monte Carlo sampling were conducted using one cold and three heated Markov chains in the analysis. Bayesian analysis was run for 645,000 generations, with trees sampled every 100 generations. The first 1290 trees, representing the burn-in phase, were discarded. To estimate posterior probabilities (PP) of recovered branches (Larget & Simon 1999) 50% majority rule consensus trees were created from the remaining trees using PAUP.

Results

Molecular phylogeny

Nucleotide sequence blasts of the NCBI-nrDNA database showed that the new fungus, *Apiosordaria hamata*, exhibited 98% LSU sequence similarity with *Cercophora* sp. isolate SMH3200 (AY780055) (query cover = 99%, identities = 1274/1295, gaps = 2/1295), 97% similarity with *Arnium arizonense* isolate 18211-c (S) (KF557668) (query cover = 100%, identities = 1252/1297, gaps

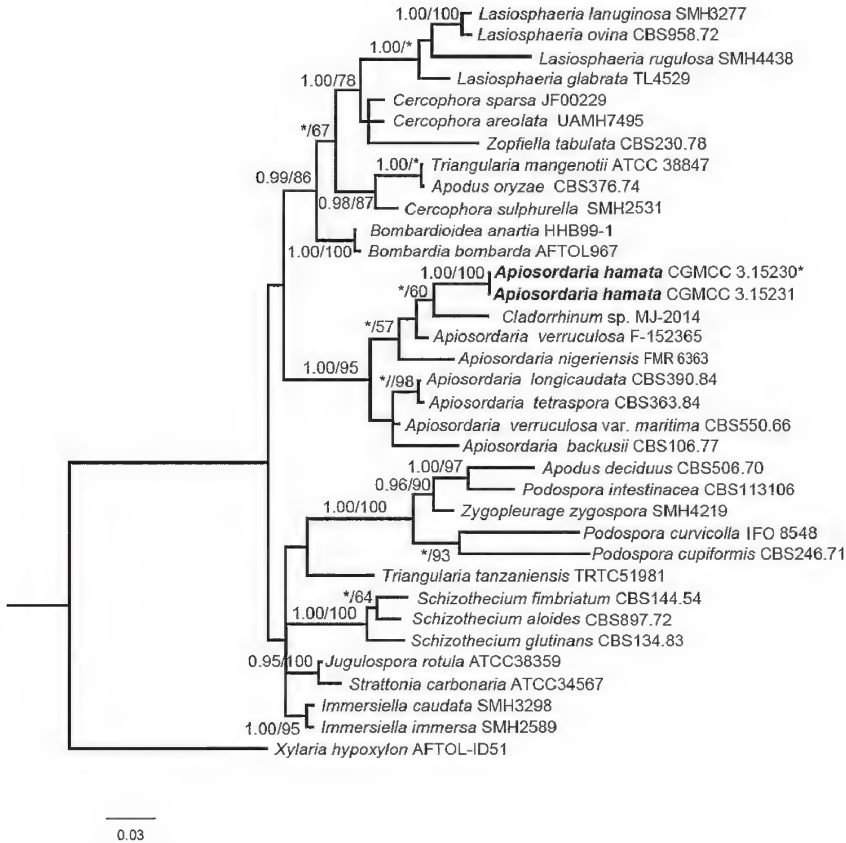


FIG. 1. Maximum likelihood (ML) tree from PAUP analysis and Bayesian tree by MrBayes based on LSU and ITS sequences of *Apiosordaria* and GenBank data from related species. Bayesian posterior probabilities >0.95 and ML bootstrap support values > 50% are shown on the upper branches.

= 3/1297), and 97% similarity with *Arniium arizonense* isolate 724 (UPS) (KF557669) (query cover = 99%, identities = 1250/1295, gaps = 3/1295) in family *Laiosphaeriaceae*. ITS sequence results indicated a 92% similarity with *Cladorrhinum* sp. MJ-2014 (KJ572182) (*Laiosphaeriaceae*) (query cover = 100%, identities = 498/544, gaps = 10/544). We therefore included related reference sequences in *Laiosphaeriaceae* in our phylogenetic analyses.

The LSU and ITS combined gene datasets were analyzed using maximum likelihood (ML) and Bayesian analysis (FIG. 1). The phylogenetic trees derived from ML and Bayesian analysis gave similar results; the *Apiosordaria hamata*

strains (CGMCC 3.15230 and CGMCC 3.15231) formed a well-supported clade with *A. backusii* (L.H. Huang) Guarro [= *Triangularia backusii*] (CBS 106.77), *A. longicaudata* (Furuya & Udagawa) J.C. Krug et al. (CBS390.84), *A. nigeriensis* Stchigel & Guarro (FMR 6363), *A. tetraspora* J.C. Krug et al. (CBS 363.84), *A. verruculosa* (C.N. Jensen) Arx & W. Gams (F-152365), *A. verruculosa* var. *maritima* (Apinis & Chesters) Arx & W. Gams (CBS 550.66), and *Cladorrhinum* sp. (MJ-2014) within *Lasiosphaeriaceae* (Bayesian posterior probability = 1; ML bootstrap value = 95%). However, the two *A. hamata* strains formed a separate clade with high support (Bayesian posterior probability = 1; ML bootstrap value = 100%).

Taxonomy

Apiosordaria hamata B. Wu, K.D. Hyde, Jing Z. Sun & Xing Z. Liu, *sp. nov.* FIG. 2

MYCOBANK MB 812280

Differs from related *Apiosordaria* species by lacking germ pores in the ascospores and by having hooked spines on the upper cell of the ascospores.

TYPE: China, Hubei, Wuhan, Donghu Lake, isolated from sediment, July 2009, B. Wu (Holotype, HMAS 246231; ex-type cultures, CGMCC 3.15230, CGMCC 3.15231).

ETYMOLOGY: *hamata* refers to the hooked spines on the upper cell of the ascospores.

FACES OF FUNGI NUMBER: FoF 00663

ASCOMATA superficial, scattered, cleistothecial, dark brown, globose, overall 475–490 × 480–525 µm. PERIDIUM 4–6-layered, up to 4–5 µm wide, translucent, brownish orange to brown, comprising cells arranged in a textura angularis. PARAPHYSES hyaline, filiform, septate, unbranched, 1.8–2.2 µm wide. ASCI 8-spored, clavate, broadly rounded at the apex, 420–450 × 90–100 µm, pedicel without conspicuous apical structures, evanescent, 90–150 µm long. ASCOSPORES biserial, clavate, hyaline, 1-celled when young and becoming two-celled with the formation of a transverse septum, warted wall, 26–31 × 22–26 µm; upper cell obovoid, truncate at the base and with a slightly acuminate apex, brown, thick-walled, uniformly ornamented with numerous 2–3 µm diam ($x = 2.4$ µm, $n = 30$) hooked spines, lacking a germ pore; lower cell subhyaline, conical and smooth, 4–7 × 12–15 µm ($x = 5.2 \times 13.1$ µm, $n = 30$) long. ASEXUAL MORPH: Undetermined.

FIG. 2. *Apiosordaria hamata* (holotype, HMAS 246231). A. colony on PCA; B, ascomata; C. immature ascoma; D, E. mature ascomata; F. immature ascus; G–I. mature asci in cotton blue; J. ascospores; K. ascospore with warted coat; L. ascospore with smooth coat; M, O. ascospores with hooked spines (SEM); N. ascoma (SEM). Scale bars: B = 1 mm; C, D = 100 µm; E = 50 µm; F–I, K, L = 10 µm; J = 20 µm.



Discussion

Apiosordaria hamata can be recognized by its spiny ascospores in the clavate asci and is supported in the phylogeny. Its ascospores are similar in size to *A. nigeriensis* (24–32 × 20–23 µm), *A. jamaicensis* (B.M. Robison) Krug et al. (24–30 × 17–23 µm), *A. sacchari* (B.M. Robison) Krug et al. (33–40 × 25–31 µm), and *A. spinosa* (Cailleux) Krug et al. (25–32 × 18–21 µm), all of which also have spinulose to spiny ascospores (Krug et al. 1983). However, *A. hamata* is distinct in having hooked spines on the upper cell of the ascospores and in lacking germ pores, which Meléndez-Howell (1970) observed in her SEM studies of previously described *Apiosordaria* species.

Phylogenetic analysis supported the two isolates of *A. hamata* in a subclade with *A. nigeriensis*, *A. verruculosa*, and *Cladorrhinum* sp. (asexual morph of *Apiosordaria*) with >50% bootstrap ML value but separated from the other *Apiosordaria* species. Although *A. hamata* is morphologically similar to *A. nigeriensis*, they are separated on the ITS and LSU phylogenetic tree (FIG. 1), which clusters *A. hamata* in a monophyletic group with seven species of *Apiosordaria*. Here, morphology coupled with molecular analysis support this fungus as a new species in *Apiosordaria* (*Lasiosphaeriaceae*).

Key to species of *Apiosordaria*

- 1. Asci 4-spored, clavate 2
- 1. Asci 8-spored, clavate or cylindrical 5
- 2. Ascomata globose, upper ascospore cell verrucose, 18–22 × 14–17 µm;
 lower ascospore cell 4–6 × 5–6 µm *A. effusa*
- 2. Ascomata pyriform, ostiolate 3
- 3. Upper ascospore cell spinulose or warty, 16–21 × 13–15 µm;
 lower ascospore cell 6–10 × 9–19 µm *A. verruculosa*
- 3. Upper ascospore cell pitted 4
- 4. Lower ascospore cell 10–14 × 7.5–9 µm *A. longicaudata*
- 4. Lower ascospore cell 4–7.5(–10) × 6–8(–11) µm *A. tetraspora*
- 5. Ascospore wall with broad pits;
 ornamentation appearing as spines or irregular warts 6
- 5. Ascospore wall with narrow pits; ornamentation appearing as pits 18
- 6. Upper ascospore cell polygonal, five-angled in side view,
 ornamented with longitudinal ribs, 10–12 × 8–9 µm *A. striatispora*
- 6. Upper ascospore cell more or less ellipsoidal 7
- 7. Ascoma ostiolate 8
- 7. Ascoma smooth or with another type of vestiture, non-ostiolate;
 ascospores overall typically larger than 14 µm 11

8. Ascoma lacking agglutinated hairs;
 upper ascospore cell 10–12.5 × 6–8 µm with flat-topped spines;
 lower cell 4–6 µm long; asexual morph absent *A. yaeyamensis*
8. Ascoma with agglutinated hairs 9
9. Upper ascospore cell 15–19 × 10–12.5 µm; lower cell 5–6.5 µm long;
 asexual morph *Chrysosporium* *A. microcarpa*
9. Upper ascospore cell larger 10
10. Ascoma pyriform; upper ascospore cell pitted, 27–34 × 18–23 µm *A. backusii*
10. Upper ascospore cell uniformly ornamented with numerous rounded warts,
 23–28(–30) × 18–22(–23) µm; lower ascospore cell 1–5(–6) µm long;
 asexual morph absent *A. hispanica*
11. Ascospores with a granulose to wart-like ornamentation;
 lower ascospore cell 4–5 µm long; asexual morph absent *A. tuberculata*
11. Ascospores with spine-like ornamentation or flat-topped ridges;
 lower ascospore cell longer 12
12. Asci cylindrical and curved; upper ascospore cell 16–20(–22) × 12–15 µm,
 lower cell frequently transversely septate, 12–20 µm long;
 asexual morphs *Cladorrhinum* and *Chrysosporium* *A. vermicularis*
12. Asci clavate; upper ascospore cell larger; lower cell non-septate 13
13. Ascospore ornamentation 2–5.5 µm long,
 upper cell (24–)25–32 × 18–21(–22) µm, lower cell 4–8 µm long;
 sexual morph *Chrysosporium* (when present) *A. spinosa*
13. Ascospore ornamentation shorter (≤3 µm); lower cell longer 14
14. Upper ascospore cell 33–40 × 25–31 µm; lower cell 14–20 µm long;
 asexual morph absent *A. sacchari*
14. Upper and lower ascospore cells shorter 15
15. Ascospore ornamentation irregular flat-topped ridges forming an incomplete
 reticulum, upper cell 28.5–33 × 17–19.5 µm; lower cell ca. 8–10 µm long;
 asexual morph *Chrysosporium* *A. terrestris*
15. Ascospore ornamentation spine-like 16
16. Upper ascospore cell without germ pore, 26–31 × 22–26 µm, uniformly
 ornamented with hooked spines of 2–3 µm diam; lower cell
 sub-hyaline, conical, smooth, 12–15 µm long; asexual morph absent *A. hamata*
16. Upper ascospore cell with germ pore 17
17. Upper ascospore cell 24–32 × 20–23 µm, with a dark area surrounding
 the germ pore and ornamented with spines ≤7 µm long;
 lower cell 7–10 µm long *A. nigeriensis*
17. Upper ascospore cell 24–30 × 17–23(–26) µm, ornamented with
 spines ≤3 µm long; lower cell ca. 11–18 µm long;
 asexual morph *Chrysosporium* (when present) *A. jamaicensis*

18. Ascoma glabrous; ascospores overall (16–)18–21(–23) × 10–12 µm, with rugose flanges; lower ascospore cell 2.5–5 µm long; asexual morph absent ... *A. rugosa*
18. Ascoma with agglutinated hairs 19
19. Upper ascospore cell regularly pitted, 20–25 × 12–15 µm, lacking flanges; lower ascospore cell 4–5 µm long; asexual morph *Cladorrhinum* *A. vestita*
19. Upper ascospore cell covered with small warts 20
20. Upper ascospore cell 27–34 × 18–23 µm, walls ornamented with shallow pits; lower ascospore cell 11–17 × 10–12 µm long *A. tenuilacunata*
20. Upper ascospore cell smaller 21
21. Asexual morph *Chrysosporium*-like; upper ascospore cell 20–24 × 15–18 µm *A. otanii*
21. Asexual morph absent 22
22. Upper ascospore cell 25–27(–29) × (22–)23–27 µm, uniformly ornamented with small warts; lower cell (1–) 4–6 µm long, slightly warted *A. globosa*
22. Upper ascospore cell 19–24 × 8–10 µm, ornamented with small warts; lower cell 3.5–4.5 × 3–4 µm *A. antarctica*

Acknowledgments

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***Discopycnothyrium palmae* gen. & sp. nov. (Asterinaceae)**

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ABSTRACT—A new genus and species, *Discopycnothyrium palmae*, is described from palms (*Arecaceae*) in Narathiwat Province, southern Thailand, and is known only from its asexual morph. The genus is characterized by circular pycnothyria with darkened cells around the central ostiole, variably long hyphae at the margin, and pycnothyriospores that are 1-septate with the septum between a larger brown upper cell and hyaline lower cell. LSU gene sequence analysis clustered *D. palmae* in the *Asterinaceae* clade and supported (59% ML and 0.88 PP support) the taxon as phylogenetically distinct from other *Asterinaceae*. Morphological and phylogenetic differences confirm the new genus, here introduced and illustrated.

KEY WORDS—*Asterinales*, foliar epiphytes, taxonomy

Introduction

The family *Asterinaceae* was introduced by Hansford (1946), with the generic type *Asterina* Lév. (Müller & von Arx 1962, Luttrell 1973, Eriksson 1981, Hosagoudar 2004, Kirk et al. 2008, Hofmann 2010, Hyde et al. 2013, Hongsanan et al. 2014). The family comprises biotrophs found on living plant leaves and is distributed worldwide (Kirk et al. 2001, Barr & Huhndorf 2001, Taylor et al., Hofmann et al. 2010, Hyde et al. 2013, Hongsanan et al. 2014). While most previous studies focused on the morphology, Hofmann (2010) provided the first molecular data of *Asterinaceae* that supported its phylogenetic

placement in *Dothideomycetes* and which were included in the phylogenetic tree representing *Asterinales* sensu stricto (Hyde et al. 2013, 2016, Wijayawardene et al. 2014). The order comprises a single family *Asterinaceae*, which contains 17 genera that are differentiated using both morphological and phylogenetic characters (Hongsanan et al. 2014). The asexual morph in the family can be either coelomycetous (*Asterostomella*) or hyphomycetous (*Asterina*), although classification of the asexual morphs needs further clarification (Hyde et al. 2013, Hongsanan et al. 2014). Redescription of the asexual morphs of *Asterinaceae* (Hofmann 2010, Hosagoudar 2010) and key to genera by Hosagoudar (2010) were based mainly on morphology and host association.

In this study, we introduce a new genus *Discopycnothyrium* and its type species *D. palmae* based on phylogeny, morphology, and host association. *Discopycnothyrium* is an asexual morph genus that somewhat resembles *Asterostomula* and *Prillieuxina* in its circular pycnothyria with radially arranged cells. However, *Asterostomula* and *Prillieuxina* differ in having pycnothyria with star-like or irregular openings and brown pycnothyriospores, while pycnothyria in *Discopycnothyrium* have a central ostiole and 1-septate pycnothyriospores comprising brown upper cells and hyaline lower cells. The placement of *Discopycnothyrium* in *Asterinaceae* is also supported by rDNA sequence analysis.

Materials & methods

An *Asterinaceae*-like specimen was collected from Narathiwat Province in the southern part of Thailand. Morphological characters were observed under Nikon 80i stereo and compound microscopes. Measurements were determined using Tarosoft (R) Image Frame Work (v. 0.9.7). We attempted to isolate the specimen by using a single spore technique (Chomnunti et al. 2011, 2014), but the spores did not grow in culture. The fresh specimen was therefore dried in silica gel, and DNA was extracted directly from pycnothyria. Type material was conserved in the Herbarium, Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand (MFLU), and the Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN).

Genomic DNA was extracted from dried pycnothyria using the E.Z.N.A® Forensic Genomic DNA Extraction Kit (OMEGA Bio-tek Norcross GA 2013). Individual pycnothyria were placed into 1.5 ml sterilized tubes and kept in -20 °C overnight, after which DNA was extracted following the manufacturer's instructions.

Universal primers LR5 and LROR were successfully used to amplify the 28S rDNA (LSU) region. The PCR thermal cycle program was: an initial cycle at 94°C for 3 min; 40 cycles of denaturation (94°C for 30 s), annealing (54°C for 40 s), elongation (72°C for 1 min); final extension (72°C for 10 min). After visualization on 1% agarose electrophoresis gels stained with ethidium bromide, the PCR products were sent to

TABLE 1. Taxa used in the phylogenetic analysis: specimen codes and GenBank accession numbers (LSU)

SPECIES	VOUCHER/CULTURE	GENBANK NO.
<i>Apiosporina collinsii</i>	CBS 118973	GU301798
<i>Asterina cesticola</i>	TH 591	GU586215
<i>Asterina fuchsiae</i>	TH 590	GU586216
<i>Asterina phenacis</i>	TH589	GU586217
<i>Asterina siphocampyli</i>	ppMP 1324	HQ701140
<i>Asterina</i> sp.	MFLU13-0619	KM386978
<i>Asterina weinmanniae</i>	TH 592	GU586218
<i>Asterina zanthoxyli</i>	TH 561	GU586219
<i>Discopycnothyrium palmae</i>	MFU13-0485	KM386979
<i>Asterotaxis cucurbitacearum</i>	PMA M-0141224	HQ610510
<i>Chaetothyriothecium elegans</i>	CPC 21375	KF268420
<i>Coleroa robertiani</i>	CBS 458.64	JQ036231
<i>Fusicladium africanum</i>	CPC 12828	EU035423
<i>Fusicladium pini</i>	CBS 463.82	EU035436
<i>Gibbera conferta</i>	CBS 191.53	GU301814
<i>Gloniopsis praelonga</i>	CBS 112415	FJ161173
<i>Gloniopsis subrugosa</i>	CBS:123346	FJ161210
<i>Hysterium angustatum</i>	CBS 236.34	FJ161180
<i>Hysterobrevium smilacis</i>	CBS 114601	FJ161174
<i>Lembosia albersii</i>	MFLU13-0377	KM386982
<i>Lichenothelia convexa</i>	L1608	KC015085
<i>Microthyrium microscopicum</i>	CBS 115976	GU301846
<i>Natipusilla limonensis</i>	L_AF286_1A	HM196370
	PE3_2b	JX474862
<i>Natipusilla naponensis</i>	L_AF217_1A	HM196371
<i>Oedohysterium insidens</i>	CBS:238.34	FJ161142
<i>Oedohysterium sinense</i>	CBS:123345	FJ161209
<i>Protoventuria barriae</i>	CBS 300.93	JQ036232
<i>Psiloglonium araucanum</i>	CBS:112412	FJ161172
<i>Psiloglonium clavisporum</i>	CBS:123338	FJ161197
<i>Psiloglonium simulans</i>	CBS:206.34	FJ161178
<i>Rhytidhysterium rufulum</i>	AFTOL-ID 2109	FJ469672
<i>Sympoventuria capensis</i>	CPC 12840	DQ885904
	CBS 120136	KF156104
<i>Tyrannosorus pinicola</i>	AFTOL-ID 1235	DQ470974
<i>Venturia inaequalis</i>	CBS 815.69	GU301878
<i>Veronaeopsis simplex</i>	CBS 588.66	EU041877
<i>Zeloasperisporium siamense</i>	IFRDCC 2194	JQ036228

¹LSU: 28S rDNA (Newly generated sequence in bold)

Majorbio Co., Ltd. (China) for purification and sequencing. The sequence generated for *Discopycnothyrium palmae* was deposited in GenBank (KM386979).

Other LSU sequence data in this study were obtained from GenBank to compile the dataset (TABLE 1), which represents *Asterinaceae*, *Hysteriaceae* Chevall., *Microthyriaceae* Sacc., *Mytiliniidiaceae* Kirschst., *Natipusillaceae* Raja et al., *Sympoventuriaceae* Y. Zhang et al., and *Venturiaceae* E. Müll. & Arx ex M.E. Barr following the publications by Schoch et al. (2009), Hyde et al. (2013), Boonmee et al. (2014), Hongsanan et al. (2014), and Wijayawardene et al. (2014). *Lichenothelia convexa* Henssen was selected as outgroup. Alignments were performed automatically using BioEdit v 7.1.9 and Clustal X 2.0.11 (Thompson et al. 1997) and improved manually when necessary (Hall 2004).

Maximum likelihood analysis was performed in RAXML with raxmlGUIv.0.9b2 (Silvestro & Michalak 2012); the search strategy was set to rapid bootstrapping and carried out using GTRGAMMAI model of nucleotide substitution for a small dataset to account for rate heterogeneity. The nucleotide substitution model was selected with MrModeltest 2.2 (Nylander 2008); the number of replicates was automatically inferred using the stopping criterion (Pattengale et al. 2009). The evolution model was performed with MrModeltest 2.2 (Nylander 2008). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes (v. 3.0b4; Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains ran for 1,000,000 generations, with trees sampled every 100th generation. 10,000 trees were obtained; the first 2000 trees, representing the burn-in phase were discarded, and the remaining 8000 trees were used to calculate posterior probabilities; the default convergence criterion was set at 0.001 (Cai et al. 2006, 2008). Phylogenetic trees were viewed in TreeView (v. 1.6.6; Page 2001). Maximum likelihood bootstrap values higher than 50% are given as the first set of numbers above the nodes (FIG. 2). Bayesian posterior probabilities (BYPP) equal or higher than 0.9 are given as the second set of numbers above the nodes (FIG. 2).

Taxonomy

Discopycnothyrium Hongsanan & K.D. Hyde, **gen. nov.**

INDEX FUNGORUM IF551024

Differs from other genera in *Asterinaceae* by its pycnothyria with darker cells surrounding a rounded central ostiole, and 1-septate pycnothyriospores with a hyaline lower cell.

TYPE SPECIES: *Discopycnothyrium palmae* Hongsanan & K.D. Hyde

ETYMOLOGY: from Latin *disco* meaning “circular” or “plate-like”, and *pycnothyrium* referring to the technical term for the type of fructification.

FACES OF FUNGI FoFoo569

Foliar epiphytes. SUPERFICIAL MYCELIUM aseptate, pale brown to brown. SEXUAL MORPH: undetermined. ASEXUAL MORPH: PYCNOTHYRIUM superficial on the host surface, circular, walls comprising radially arranged cells, dark brown, with central ostiole surrounded by dark cells. PERIDIUM poorly

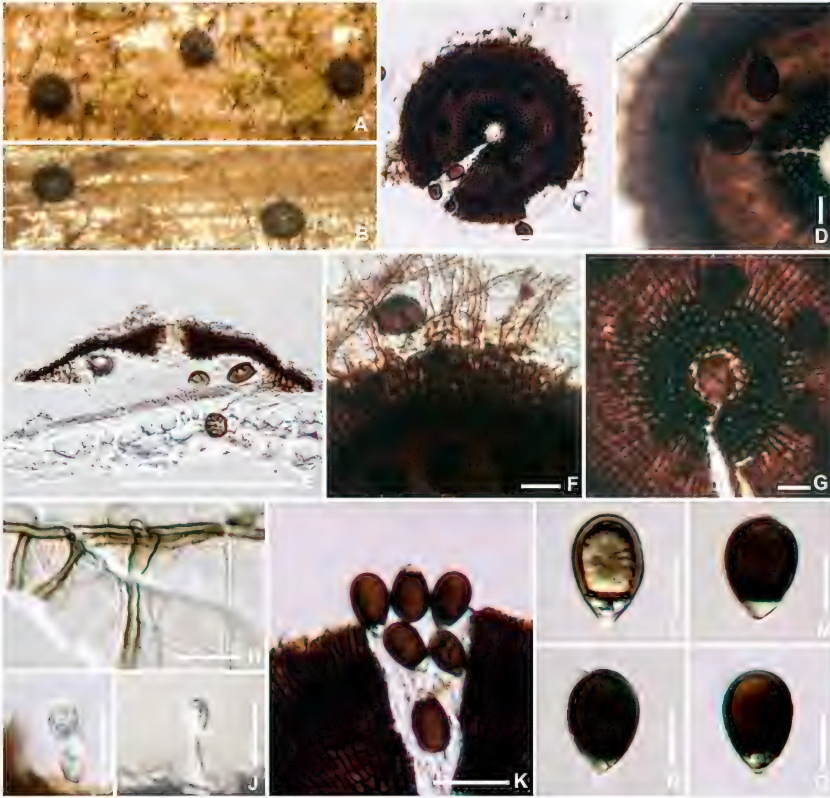


FIG 1. *Discopycnothyrium palmae* (MFLU 13-0485, holotype). A, B. Pycnothyria on surface of host. C. Pycnothyrium when viewed in squash mounts. D. Pycnothyriospore arrangement in pycnothyrium. E. Vertical section through pycnothyrium. F. Upper wall of pycnothyrium when viewed in squash mounts. G. Darkened cells around ostiole. H. Aseptate hyphae without appressoria. I, J. Conidiogenous cells giving rise to pycnothyriospores. K, M–O. Mature pycnothyriospore. L. Immature pycnothyriospore. Bars: C–E = 100 μ m; K = 20 μ m; D, F–J, L–O = 10 μ m.

developed at the base. CONIDIOGENOUS CELLS holoblastic, cylindrical, hyaline. PYCNOTHYRIOSPORES ovoid or broadly clavate, widest and rounded near the apex and tapering towards lower end, 1-septate near the tapering base, upper cell pale brown to brown, lower cell hyaline.

Discopycnothyrium palmae Hongsanan & K.D. Hyde, sp. nov.

FIG. 1

INDEX FUNGORUM IF551023

Differs from other species in *Asterinaceae* by its darker brown cells surrounding a rounded central ostiole, and its 1-septate pycnothyriospores with a hyaline lower cell.

TYPE: Thailand, Narathiwat Province, on the branches of palm (*Arecaceae*), September 2013, K.D. Hyde (**holotype** MFLU13-0485; **isotype** KUN; GenBank KM386979).

ETYMOLOGY: Referring to the host palm on which the fungus was found.

FACES OF FUNGI FoFo0570

Foliar epiphytes on surface of palm leaves (*Arecaceae*). SUPERFICIAL MYCELIUM 1–2 μm diam ($x = 2 \mu\text{m}$, $n = 10$), aseptate, pale brown to brown, dark brown at the margin, lacking appressoria and setae.

SEXUAL MORPH: Undetermined.

ASEXUAL MORPH: PYCNOTHYRIUM 162–195 μm diam ($x = 187 \mu\text{m}$, $n = 10$), superficial on host surface, easily removed, mostly solitary, circular, hyphae of different lengths at the margin, wall comprising radial cells, dark brown, with central ostiole, and with darker cells around the central ostiole. PERIDIUM poorly developed at the base. PSEUDOPARAPHYSES not observed. CONIDIOPHORES not observed. CONIDIogenous CELLS $6\text{--}8 \times 2\text{--}3 \mu\text{m}$ ($x = 8 \times 3 \mu\text{m}$, $n = 5$), holoblastic in cavity of pycnothyria, cylindrical, hyaline, smooth. PYCNOTHYRIOSPORES $18\text{--}22 \times 12\text{--}15 \mu\text{m}$ ($x = 20 \times 13 \mu\text{m}$, $n = 10$), ovoid or broadly clavate, widest and rounded near the apex and tapering towards the lower end, 1-septate near the tapering base, not constricted at the septum, upper cell with 2 layers when immature, outer layer disappearing at maturity, pale brown in upper cell when immature and dark brown at maturity with smooth-walls, lower cell always hyaline, smooth-walled or sometimes rough.

NOTES: *Discopycnothyrium palmae* is most similar to *Asterostomula loranthei* Theiss. by having a superficial mycelium without appressoria and setae and pycnothyrium walls composed of radial cells, but *A. loranthei* differs in the stellate dehiscence of the pycnothyrium centers and its unicellular pycnothyriospores (Hosagoudar 2010).

The new species also somewhat resembles the asexual morph of *Prillieuxina*, which also has circular pycnothyria and dark pycnothyriospores with a single septum, and a similar host association (Hofmann 2010), with *P. calami* (Syd. & P. Syd.) R.W. Ryan and *P. saginata* (Syd. & P. Syd.) R.W. Ryan also described from areaceous hosts. *Prillieuxina* differs from *Discopycnothyrium* by its circular pycnothyria with X- or Y-shaped fissures at maturity and its 1-septate pycnothyriospores with a brown (not hyaline) lower cell (Hongsanan et al. 2014).

Allothyrium marcgraviae Syd. also has circular pycnothyria but differs from *D. palmae* by the star-like fissures of its pycnothyrial opening and its multi-septate ascospores (Hongsanan et al. 2014). The LSU sequence analyses cluster *D. palmae* in the clade of *Asterinaceae* but in its own lineage, with 59% ML and 0.88 PP support (FIG. 2), confirming it as a new genus.

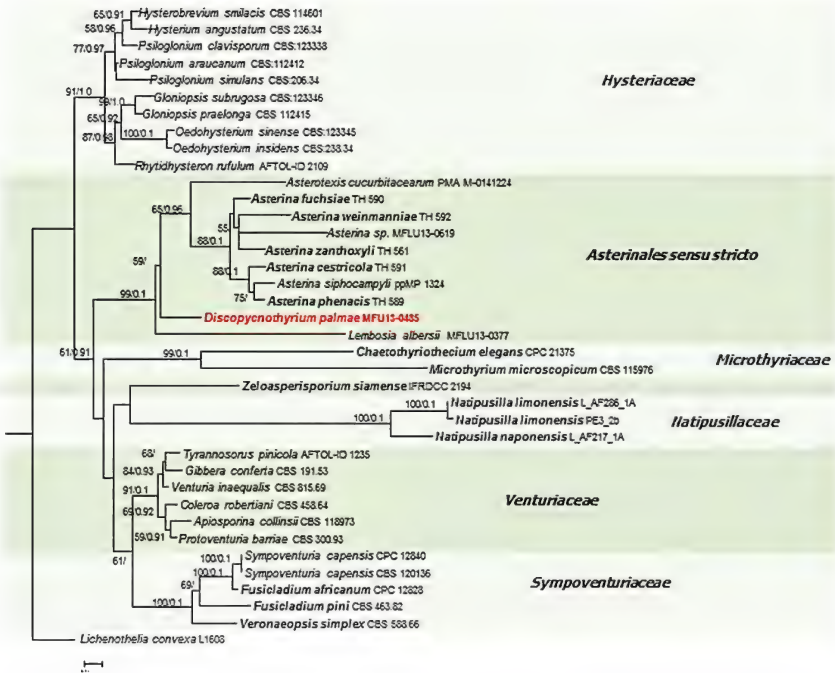


FIG 2. RAxML maximum likelihood phylogenetic tree based on sequence analysis of LSU dataset. The first set of numbers above the nodes are RAxML values >50%; the second set are Bayesian posterior probabilities ≥0.9. Strain numbers are indicated after species names. The new species introduced is in red bold, other types are in black bold.

Molecular phylogeny

LSU sequence data from six families (*Hysteriaceae*, *Microthyriaceae*, *Mytiliniaceae*, *Natipusillaceae*, *Sympoventuriaceae*, *Venturiaceae*) were included in the phylogenetic analysis (FIG. 2). The *Asterinaceae* clade includes six strains of *Asterina*: *A. cesticola* (R.W. Ryan) Hosag. & T.K. Abraham, *A. fuchsiae* Syd., *A. phenacis* Syd., *A. siphocampyli* Syd., *A. weinmanniae* Syd., and *A. zanthoxyli* W. Yamam. The new taxon, *Discopycnothyrium palmae*, forms a moderately supported (59% ML, 0.88 PP) clade sister to the other *Asterina* species, supporting *D. palmae* as clearly separate from other species in *Asterinaceae*. Moreover, *Lembosia albersii* Henn. (MFLU13-0377) forms a well-supported clade basal to the other genera in *Asterinaceae* with high (99% ML, 1.0 PP) bootstrap support.

The *Microthyriaceae* clade comprises two strains: *Chaetothyriothecium elegans* Hongsanan & K.D. Hyde and *Microthyrium microscopicum* Desm.,

also with high (99% ML, 1.0 PP) bootstrap support. The *Natipusillaceae* clade includes three strains of freshwater fungi: *Natipusilla limonensis* A. Ferrer et al. (two strains) and *N. naponensis* A. Ferrer et al. with 100% ML and 1.0 PP support; although they are phylogenetically related to *Zeloasperisporium siamense* Hongsanan et al., there is no morphological similarity between *Natipusilla* and *Zeloasperisporium*, so these should be regarded as tentatively placed lineages.

The *Venturiaceae* clade) with high bootstrap support of 91% ML, 1.0 PP) includes six representative strains—*Apiosporina collinsii*(Schwein.) Höhn., *Coleroa robertiani* (Fr.) E. Müll., *Gibbera conferta* (Fr.) Petr., *Protoventuria barriar*e Carris & A.P. Poole, *Tyrannosorus pinicola* (Petrini & P.J. Fisher) Unter. & Malloch, and *Venturia inaequalis* (Cooke) G. Winter. The clade of *Venturiaceae*—and is closely (66% ML support) related to the *Sympoventuriaceae* clade with five strains: *Sympoventuria capensis* Crous & Seifert (two strains), *Fusicladium africanum* Crous, *F. pini* Crous & de Hoog, and *Veronaepsis simplex*(Papendorf) Arzanlou & Crous.

Discussion

Discopycnothyrium (type species *D. palmae*) is introduced as a new genus in the family *Asterinaceae* (characterised by a superficial pycnothyrium comprising radiating cells) that differs from other genera in having circular pycnothyria with variably long marginal hyphae, darkened cells around a central ostiole, and pycnothyriospores that are widest near the apex, taper towards lower end, and have one septum near the tapering base that separates the larger pale brown to brown upper cell from the smaller hyaline basal cell.

There are limited sequence data for taxa in *Asterinaceae* because members of this family are mostly obligate parasites/biotrophs, and pure cultures are difficult to obtain; therefore direct DNA extraction and sequencing must be obtained from specimen. However, sequence data from *Asterina* species do support the *Asterinaceae* within *Dothideomycetes* (Hofmann 2010, Wu et al. 2011, Hyde et al. 2013, Boonmee et al. 2014, Hongsanan et al. 2014, Wijayawardene et al. 2014). Although phylogenetic LSU analyses of *Discopycnothyrium* cluster *D. palmae* within the *Asterinaceae* clade, it is distinctly separate from other genera in that clade. Bootstrap values are not high because of low number of populations representing this group, and further study is needed to clarify the divergence of the species. Based on

Nonetheless, both morphology and phylogeny supports the new taxon as a species in a new genus within *Asterinaceae*, which we introduce here as *Discopycnothyrium*.

Acknowledgments

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Two new records of *Agaricus* from Southwest China

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ABSTRACT—Two specimens of *A. brunneosquamulosus* (from China and Thailand) and four specimens of *A. duplocingulatus* (from China) were identified and studied by morphological and ITS sequence analyses. They are presented here with descriptions, line drawings, and color photographs as new records for China. Our additional molecular sampling of *A. duplocingulatus* has revealed intraspecific variability within this species.

KEY WORDS—Agaricaceae, rDNA, taxonomy

Introduction

Agaricus L. is a well-known genus containing species with a high nutritional and medicinal value, such as the famous cultivated mushrooms *A. bisporus* (J.E. Lange) Imbach and *A. subrufescens* Peck. Zhao et al. (2011) recognized 386 species in this cosmopolitan genus, a number that has subsequently risen to over 400 with the discovery of numerous novel taxa from Asia, Australia, and Europe (Chen et al. 2012, Gui et al. 2015, Lebel 2013, Lebel & Syme 2012, Li et al. 2014, Parra 2013, Zhao et al. 2012). Eight sections were traditionally recognized in the genus, long classified based on temperate species (Challen et al. 2003; Kerrigan et al. 2005; Kerrigan et al. 2008; Parra 2008, 2013). However, following a phylogenetic study of samples from both temperate and tropical

regions by Zhao et al. (2011), eleven new lineages comprising tropical or subtropical taxa were revealed.

One new clade determined by Zhao et al. (2011) has been phylogenetically reconstructed and identified as corresponding with *Agaricus* sect. *Brunneopicti* Heinem. (Chen et al. 2015). The section has been delimited to accommodate species that combine the following morphological characters: punctiform squamulose remnants of the universal veil or brownish larger squamules on pileus surface; a double or complex annulus with cortinate fibrils on the under side; context when bruised discoloring faint to strong yellow, orange, rufescent, brownish rufescent, or red; odor of bitter almond, phenol, or solvent; a positive KOH reaction, and a negative or (rarely) weakly positive Schäffer's reaction (Chen et al. 2015). The section, which has a strictly palaeotropical distribution, contains 16 species.

Southwestern China is known as home to glacial refugia and plays an important role in postglacial biological recolonization. In order to reveal the species spreading patterns and biodiversity of *Agaricus*, since 2010 we have conducted a series of investigations in this area during the July–August rainy season. As one result, two representatives of *A.* sect. *Brunneopicti*—*Agaricus brunneosquamulosus* and *A. duplocingulatus*—are newly recorded from China.

Materials & methods

Morphological study

Specimens were collected in Yunnan Province, China, during the rainy season. Photographs were taken immediately in the field. Each specimen was wrapped in aluminum foil or kept separate in a plastic collection box. Macroscopic characters were recorded and chemical tests were performed immediately after the specimens reached the laboratory according to Largent (1986). The specimens were dried completely at 70°C in a food drier, sealed in plastic bags, and deposited in the herbarium of Southwest Forestry University, Kunming, China (SWFC), with duplicates in Herbarium, Mycologicum Academiae Sinicae, Beijing, China (HMAS). An additional specimen from Thailand (deposited in HMAS) was included in the study.

Following the methodology described by Largent (1986), microscopical features (basidiospores, basidia, cystidia, pileipellis, annulus hyphae) of each dried specimen were examined. At least 20 measurements were made to calculate the dimensions presented as follows: X = the means $\pm$ SDs of length $\times$ width, Q = basidiospore length : width ratio, and Q_m = the mean $\pm$ SD of Q values.

DNA extraction, PCR, and sequencing

DNA was extracted from the dried fruiting bodies using the E.Z.N.A. Forensic DNA Extraction Kit (D3591-01, Omega Bio-Tek). The internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS5 following the protocol of White et al. (1990). DNA sequencing was performed in commercial biotechnology company.

TABLE 1. *Agaricus* specimens included in the phylogenetic analyses.

SPECIES	SAMPLE	ORIGIN	ITS SEQUENCE
<i>A. sp. 1</i>	C3182	Togo	KJ540955
<i>A. sp. 2</i>	LD2011027	Thailand	KJ540955
<i>A. sp. 3</i>	ZRLwxh3115	China	KR812336
<i>A. bingensis</i>	ADK1992	Bénin	KJ540954
	C3155	Togo	KJ540950
<i>A. niveogranulatus</i>	LD2011025	Thailand	KJ540957
	LD2011023	Thailand	KJ540958
	LD2011024	Thailand	KJ540959
	PYP009	Thailand	KJ540960
<i>A. chiangmaiensis</i>	NTS113	Thailand	JF514513
<i>A. brunneopunctatus</i>	ADK2564	Bénin	JF514518
<i>A. toluenolens</i>	CA911	Thailand	KJ540947
	CA926	Thailand	KJ540948
<i>A. sp. 4</i>	NTT117	Thailand	JF514534
<i>A. sordidocarpus</i>	LD201237	Thailand	KJ540946
<i>A. subsaharianus</i>	ADK4732	Burkina-Faso	JF440300
<i>A. sp. 5</i>	CA800	Thailand	JF727826
<i>A. megacysidiatus</i>	LD2012168	Thailand	KF305947
	LD2012179	Thailand	KF305946
	NTS115	Thailand	KC971098
	NTS116	Thailand	JF514532
	NT019	Thailand	JF727844
<i>A. sp. 6</i>	NTT118	Thailand	KJ540970
<i>A. brunneosquamulosus</i>	LD201238	Thailand	KJ540969
	ZRL3005	Thailand	KR869107
	ZRL4017	Thailand	JF691549
	LD2012105	Thailand	KJ540968
	ZRL133	Thailand	KR812337
	ZRL2013266	China	KR812340
<i>A. cf. inoxydabilis</i>	LAPAF1	Togo	JF727841
<i>A. duplocingulatus</i>	ZRL3064	Thailand	KR869108
	CA903	Thailand	KJ540962
	LD2012177	Thailand	KJ540961
	LD201218	Thailand	KJ540967
	LD201233	Thailand	KJ540965
	LD201274	Thailand	KJ540963
	LD201275	Thailand	KJ540964
	ZRL3031	Thailand	JF691550
	ZRL3041	Thailand	KR869109
	NTT34	Thailand	JF514536
	ZRL2013328	China	KR812341
	ZRL2012267	China	KR812339
	ZRL2012120	China	KR812338
	ZRL2013348	China	KR812342

Phylogenetic analyses

Our original sequences plus those retrieved from GenBank (TABLE.1) were first aligned using ClustalX 2.0 (Thompson et al. 1997) and then manually adjusted in BioEdit v.7.0.4 (Hall 2007). Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). One million generations using a GTR+I+G model nucleotide substitution detected by MrModeltest 2.2 (Nylander 2004) were run for six Markov chains and sampled every 100th generation resulting in 10,000 trees. Maximum Parsimony analysis was performed using PAUP*4.0b10 (Swofford 2004). One thousand heuristic searches were conducted with random sequence addition, tree bisection-reconnection (TBR) branch swapping, and gaps treated as missing data. Parsimony bootstrap values were obtained from 1000 bootstrap replicates, with starting trees obtained via stepwise addition, random sequence addition, and max-trees set to 1,000,000.

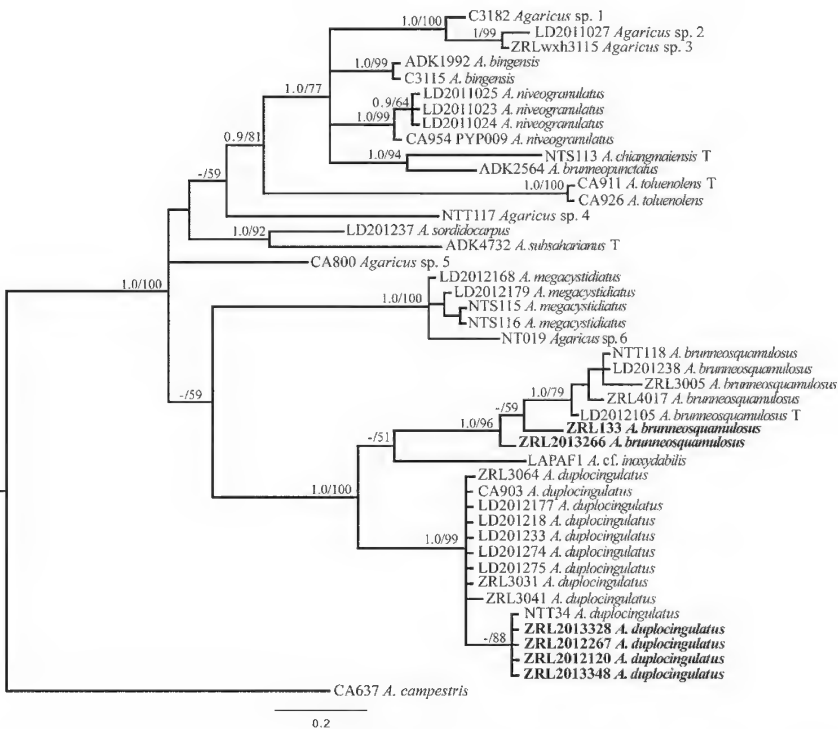


FIGURE 1. Phylogeny of *Agaricus* sect. *Brunneopicti* generated from MrBayes analysis of ITS sequences rooted with *A. campestris*. New sequences are set in bold font. Bayesian posterior probability (PP) values >0.9, and parsimony bootstrap support (BS) values >50% are given at the nodes.

Results

Phylogenetics analyses

The dataset comprised 45 sequences representing 17 species with *A. campestris* as outgroup (Zhao et al. 2011). The final alignment comprised a total of 663 characters, of which 517 were constant, 39 parsimony-uninformative, and 107 parsimony-informative. The phylogenetic trees generated by MP and Bayesian methods exhibited very similar topologies, with the Bayesian tree shown in FIG.1. Collections ZRL2013266 (from China) and ZRL133 (from Thailand) clustered in the *A. brunneosquamulosus* clade with strong support (1/96); and Chinese collections ZRL2013328, ZRL2012267, ZRL2012120, and ZRL2013348 grouped with NTT34 (from Thailand; Zhao et al. 2011) in the *A. duplocingulatus* clade with strong support (1/99).

Taxonomy

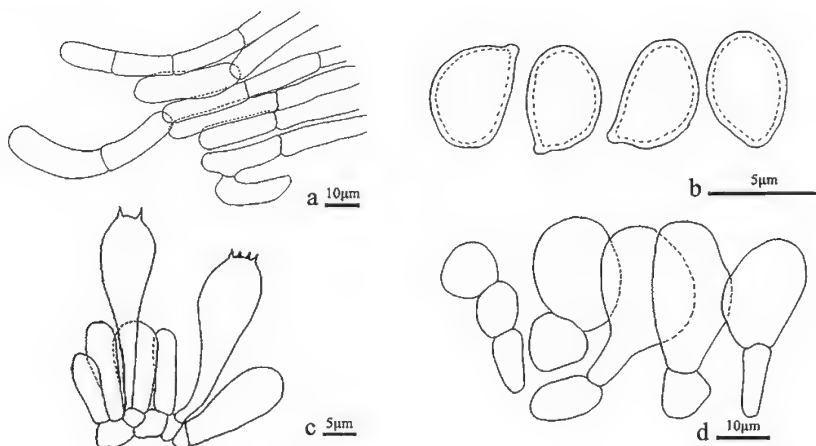


FIGURE 2. *Agaricus brunneosquamulosus* (ZRL2013266):
a. Pileipellis; b. Basidiospores; c. Basidia; d. Cheilocystidia.

Agaricus brunneosquamulosus L.J. Chen, R.L. Zhao, K.D. Hyde & Callac,

Phytotaxa 192(3): 154 (2015).

FIGS 2, 4A,B

Macroscopical characters: PILEUS 26 mm in diam, parabolic, convex, margin decurved; surface dry, with appressed triangular fibrillose squamules, light brown to brown against white background; LAMELLAE free, crowded, pink to brown, 3 mm broad, with 3 series of lamellulae. STIPE 60 × 8–16 mm (at base), thickening towards the base, with long rhizomorphs; surface white, covered by fine fibrils; context narrow, hollow. ANNULUS double (two adhering layers): upper layer a membranous remnant of the partial veil, rigid, pendant, white,

≤21 mm diam, 3 mm thick; bottom layer a fibrillose-membranous remnant of the universal veil, fragile; white above and with brown fibrils below. CONTEXT 3 mm thick, white; discoloration indistinct when touched, none when cut. Odor not noted.

Macrochemical reactions: KOH—slightly yellow. Schäffer's reaction—negative.

Microscopical characters: BASIDIOSPORES $4.1\text{--}5.6 \times 2.8\text{--}3.7 \mu\text{m}$, [$X = 4.8 \pm 0.4 \times 3.3 \pm 0.3 \mu\text{m}$, $Q = 1.21\text{--}1.65$, $Q_m = 1.47 \pm 0.13$, $n = 20$], ellipsoid, smooth, brown, thick-walled. BASIDIA 4-spored, $18.5\text{--}30.6 \times 5.9\text{--}8.7 \mu\text{m}$, narrowly clavate to clavate, hyaline, smooth. CHEILOCYSTIDIA $14.6\text{--}27.6 \times 10.9\text{--}25.3 \mu\text{m}$, pyriform, clavate to broadly clavate, basally septate or catenulate with 2–3 ellipsoid to subspherical cells in chain, hyaline, smooth. PLEUROCYSTIDIA absent. PILEIPELLIS a cutis, hyphae $4.3\text{--}11.5 \mu\text{m}$ diam, cylindrical, light brown, smooth, sometimes slightly constricted at the septa.

HABIT: Solitary in forest.

SPECIMENS EXAMINED: CHINA, YUNNAN PROVINCE, Teng Chong County, Laifeng Mountain, 18 July 2013, Zhou Junliang, ZRL2013266 (HMAS, SWFC; GenBank KR812340). THAILAND, CHIANG MAI PROVINCE, Mae Taeng District, Ban Pha Deng Village, Mushroom Research Centre, 15 May 2008, Zhao Ruilin, ZRL133 (HMAS; GenBank KR812337).

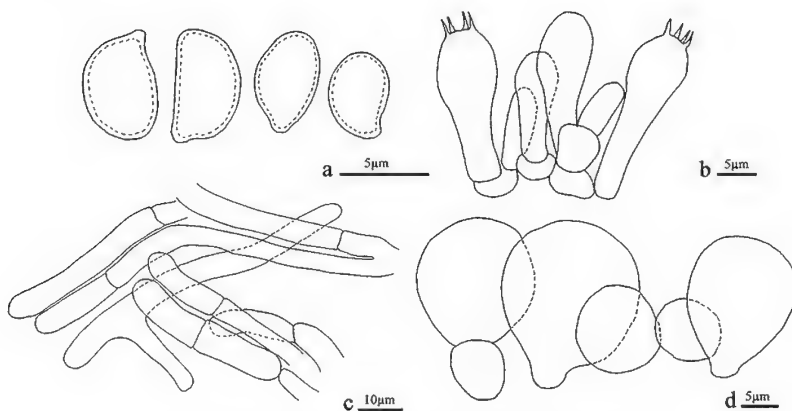


FIGURE 3. *Agaricus duplocingulatus* (ZRL2013328):
a. Basidiospore; b. Basidia; c. Pileipellis; d. Cheilocystidia.

Agaricus duplocingulatus Heinem., Bull. Jard. Bot. Natl. Belg. 50: 32 (1980). FIGS 3, 4C–H.

Macroscopical characters: PILEUS 27–87 mm diam, convex, margin decurved when young, becoming straight when mature; surface dry, covered



FIGURE 4. *A. brunneosquamulosus* (a, b: ZRL2013266). Habit.
A. duplocingulatus (c, d: ZRL2012120; e, f: ZRL2012267; g, h: ZRL2013328). Habit.

with reddish brown appressed fibrillose squamules on a white background; squamules congregated on the disc and concentrically arranged elsewhere; fibrillose or mud-cracked [areolate?]; surface becoming dull red when wet. LAMELLAE free, crowded, lamellulae in 5 tiers, 1–6 mm broad, pink when young, brown when mature. STIPE 30–43 × 4–9 mm, cylindrical with enlarged (4–23 mm diam) base, with rhizomorphs, hollow; surface silky; white, turning reddish brown when bruised or cut. ANNULUS double, two separate layers: upper layer wider (47 mm diam), persistent, membranous, white, smooth above and fibrillose to floccose below; lower layer, narrower (12 mm diam), bracelet-like, fugacious, movable, white and floccose above, smooth with a brown margin below. CONTEXT 4 mm broad, firm; discoloration variable: distinctly yellow when touched and red when cut or no discoloration when touched or cut. Odor almond-like.

Macrochemical reactions: KOH reaction yellow. Schäffer's reaction negative.

Microscopical characters: BASIDIOSPORES 4.5–6.5 × 3.6–4.5 μm [$X = 5.7 \pm 0.3 \times 4.0 \pm 0.2 \mu\text{m}$, $Q = 1.30\text{--}1.7$, $Q_m = 1.40 \pm 0.10$, $n = 20$], ellipsoid, smooth, brown, thick-walled. BASIDIA 4-spored, 14.5–19.3 × 4.9–7.5 μm ,

clavate, hyaline, smooth. CHEILOCYSTIDIA $14.2\text{--}27.3 \times 8.1\text{--}19.6\text{ }\mu\text{m}$, globular to pyriform, hyaline, smooth. PLEUROCYSTIDIA absent. PILEIPELLIS a cutis, hyphae $2.3\text{--}7.0\text{ }\mu\text{m}$ diam, cylindrical, light brown, smooth, slightly constricted at the septa.

HABIT: Solitary in forest.

SPECIMENS EXAMINED: CHINA, YUNNAN PROVINCE, Ying Jiang County, Tong Biguan National Natural Reserve, 19 July 2013, Yu Qinghua, ZRL2013328 (HMAS, SWFC; GenBank KR812341); 20 July 2013, Yu Qinghua, ZRL2013348 (HMAS, SWFC; GenBank KR812342); Cang Yuan county, Nan Gunhe National Natural Reserve, 7 July 2012, Zhao Ruilin, ZRL2012120 (HMAS, SWFC; GenBank KR812338); An Kang Village, 11 July 2012, Philippe Callac, ZRL2012267 (HMAS, SWFC; GenBank KR812339).

Discussion

Agaricus brunneosquamulosus, originally described from Thailand, is characterized by small sporocarps, tiny ferruginous brown appressed triangular squamules on the pileus surface, and a complex annulus with woolly scales and cortinate fibrils on its lower surface (Chen et al. 2015). The Chinese material generally agrees with original description, except for the uncertain annulus morphology of annulus and occasional presence of short catenulate cheilocystidia. These annular and cheilocystidial differences may be due to the immaturity of the Chinese collection. The ITS sequence alignment does indeed show a five nucleotide difference between the Thai and Chinese collections; however, all the differences are transition mutations (T/C or A/G), polymorphisms commonly observed in intraspecific variation (Zhang et al. 2009, Yadav 2014). In the absence of significant morphological differences between the Thai and Chinese material, we for the time recognize the Chinese material as *A. brunneosquamulosus*. Collection of more young to mature materials in near future will undoubtedly refine our determination.

Agaricus duplocingulatus, first described from Singapore and recently reported from Thailand, is characterized by brown ferruginous appressed squamules on the pileus surface, a double annulus with the lower one movable, an ochraceous to reddish discoloration when cut or bruised, and catenulate cheilocystidia (Heinemann 1980, Chen et al. 2015). The four Chinese collections, which generally match the species circumscription, are more variable in sporocarp size (pileus 27–87 mm diam). Comparison of the ITS sequences reveals nucleotides that differ at four polymorphic positions in more than 16 positions, also by Chen et al. (2015). This degree of intraspecific variability is rarely observed in *Agaricus*, and multi-gene analysis may be helpful in determining whether *A. duplocingulatus* represents a complex of species. Presently, we adopt the identification by Chen et al. (2015).

Acknowledgements

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***Entoloma ochreoprunuloides* from Italy, with notes on its geographical distribution and allied species**

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ABSTRACT—An Italian collection of *E. ochreoprunuloides* [$\equiv$ *E. prunuloides* var. *obscurum*] is described. The specimen was identified by means of morphology, and by the analysis of its nrITS sequence. The European distribution of the species is also discussed. The sequence from a single Italian specimen of *E. luteobasis* suggests that *E. luteobasis* and *E. ochreoprunuloides* may be conspecific.

KEY WORDS—*Basidiomycota*, *Agaricomycetes*, tricholomatoid clade, taxonomy

Introduction

Entoloma (Fr. ex Rabenh.) P. Kumm. is a large genus of *Agaricomycetes*, with worldwide distribution; more than 1500 taxa have been described (Morgado et al. 2013). Until a few years ago, the systematics of *Entoloma* was based only on morphological data (Noordeloos 1981, 1992, 2004), but recent studies based on DNA sequences have started to appear in the literature (e.g., Co-David et al. 2009; Baroni et al. 2011; Kokkonen 2015). The consequence of these investigations is that the infrageneric subdivision is continuously evolving, but this process is just at the beginning. For example, Noordeloos (1981, 1992, 2004) treats *Entoloma* as a single genus subdivided into several subgenera, while Largent (1994) recognizes *Entoloma* sensu stricto and many segregate genera,

most corresponding to the subgenera used by Noordeloos. Recently Baroni et al. (2011) proposed *Entocybe* T.J. Baroni et al. as a new genus that includes a few select species from *Entoloma* subg. *Entoloma* and a few other anomalous species previously placed in *Rhodocybe* sect. *Rhodophana* (Kühner) Singer. These taxa produce bumpy spores reminiscent of those found in *Rhodocybe* and abundant clamp connections that are not typical of *Rhodocybe*. *Entocybe* was erected for a distinct clade in *Entoloma* subg. *Entoloma* based on both morphological and molecular data.

At the species level, even some well-known species appear far more complex when investigated at the molecular level. For example, Morgado et al. (2013) focused on the subgenus *Entoloma* using a multigene approach (mtSSU, nrLSU, rpb2, nrITS) to find that *E. bloxamii* (Berk. & Broome) Sacc., *E. prunuloides* (Fr.) Quél., and *E. sinuatum* (Bull.) P. Kumm. each represent species complexes containing different taxa when considered from a worldwide perspective. Kokkonen (2015), who examined nrITS, nrLSU and rpb2 sequences of several *Entoloma* taxa mainly in *E.* sect. *Rhodopolia* (Fr.) Noordel. (actually elevated at subgeneric level), also proposed several novel taxa and combinations.

The present contribution concerns the *E. prunuloides* complex in *E.* sect. *Entoloma*, a section characterised by small, somewhat isodiametric and obscurely angular spores and pileipellis hyphae arranged as a simple cutis or ixocutis. From a phylogenetic point of view, the species of this section form a clade that appears basal to the bulk of the species in the *Entoloma* sensu lato clade. Morgado et al. (2013) demonstrated that at least three different species have been covered under the name “*E. prunuloides*”—(1) *E. pseudoprunuloides* Morgado & Noordel., for a Canadian collection; (2) typical *E. prunuloides*, probably with an exclusively European distribution; and (3) *E. ochreoprunuloides* [= *E. prunuloides* var. *obscurum*], from France, Germany, and United Kingdom.

We here describe the finding of an Italian collection of *E. ochreoprunuloides*, whose nrITS sequence was examined and confirmed as conspecific with the holotype collection.

Materials & methods

Morphology

Fresh basidiomata were photographed in the field using a Nikon D80 digital camera. Microscopic features were examined using a Nikon Eclipse E200 light microscope and observations were made on mounts in Congo red, followed by 5% NH₃ solution.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from two herbarium specimens (*E. ochreoprunuloides* TO 3327; *E. luteobasis* WU 17842) with NaOH (Osmundson et al. 2013); 2 mg of dry sample were homogenized in 250 µl of soda at 0.5M with a pestle. After 5 minutes to allow for

sedimentation, 5 µl of the extract were removed and diluted in 195 µl of 100mM Tris-HCl at pH 8.0, and 1 µl of the dilution was used as template DNA. The nrITS region was amplified with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR was performed in 25 µl reaction volumes following Gardes & Bruns (1993).

PCR products were purified and sequenced by IGA Technology Services (Udine, Italy).

Results

The ITS sequence of our Italian collection (TO 3327; GenBank KU984720) shared 99% (582/583) of nucleotides with the German holotype of *E. ochreoprunuloides* (L [E. Arnolds 01-142]; GenBank KC710092) and 100% homology with two independent sequences from an Italian collection labelled as *E. luteobasis* (WU 17842; GenBank KU984721 [this paper], LN850613 [Kokkonen 2015]).

Taxonomy

Entoloma ochreoprunuloides Morgado & Noordel., Persoonia 31: 171. 2013.

FIGS 1A, C–F

≡ *Entoloma prunuloides* var. *obscurum* Arnolds & Noordel., Fungi Europaei
vol. 5a: 838. 2005 [non *Entoloma obscurum* Hesler 1967].

SELECTED ICONOGRAPHY: Noordeloos (2004; Pl. 1b, p. 1166), Morgado et al. (2013; Fig. g 1–2, p. 168)

Description of the Tuscan collection (TO 3327)

PILEUS 4–6.5 cm broad, convex, or even conico-convex when young, then more applanate but with a consistently broad (sometimes acute) central umbo; surface fibrillose (even strongly so), greasy; colour sepia brown to ochre, darker in the center, lighter and more brown-yellow at the periphery; hygrophane; margin irregular, fimbriate, strongly involute, striate. **LAMELLAE** emarginate to almost adnate, crowded, at first whitish or light grey, then pink. **STIPE** 5–9 × 0.5–1.4 cm, cylindrical, base ending in a distinctly tapered tip; longitudinally fibrillose, concolourous with the pileus, whitish towards base. **CONTEXT** rather thin in the pileus, thicker near the umbo, whitish; **SMELL** intensely mealy (farinaceous), **TASTE** mealy.

SPORES 6.0–7.6 × 5.4–7.1 µm, av. 6.8 × 6.4 µm, $Q = 1.0\text{--}1.17(-1.26)$, $Q_{av} = 1.08$, somewhat isodiametric, 4–5(–6)-angled, angles blunt under the light microscope. **BASIDIA** 27–40 × 7–11 µm, 4-spored, clavate. **HYMENOPHORAL TRAMA** made up of cylindrical to fusoid, parallel hyphae, 35–60 × 4–8 µm. **PILEIPELLIS** three-layered, with the outermost layer (suprapellis) an ixocutis of very narrow (1.5–2.5 µm diam) hyphae dispersed in a gelatinous matrix, the middle layer (mediopellis) a cutis of parallel cylindrical hyphae (4–6 µm

diam), and the bottom layer (subpellis) of broad, inflated hyphae measuring $25\text{--}50 \times 15\text{--}25\ \mu\text{m}$. Pigments pale, intracellular, diffuse. STIPITPELLIS a cutis of narrow ($3\text{--}5\ \mu\text{m}$ diam) cylindrical hyphae. CAULOCYSTIDIA absent. CLAMP-CONNECTIONS frequent in all tissues.

HABITAT densely gregarious to sub-cespitose in a broad-leaved wood composed mainly of *Quercus cerris* L. (Turkey oak; *Fagaceae*) and *Carpinus betulus* L. (hornbeam; *Betulaceae*).

MATERIAL STUDIED: *Entoloma ochreoprunuloides*: ITALY, TUSCANY, Grosseto, Scansano, Monte Auto, under *Quercus cerris* and *Carpinus betulus*, 6.11.2013, legit M. Clericuzio (TO 3327; GenBank KU984720).

ADDITIONAL MATERIAL STUDIED: *Entoloma luteobasis*: ITALY, APULIA, Foggia, Vieste, Val del Tesaro, under *Q. cerris*, 16.11.1997, legit A. Hausknecht & F. Reinwald (WU 17842; GenBank KU984721)

Discussion

The Italian collection of *E. ochreoprunuloides* was made by one of us (MC) in Tuscany (Italy) in a mixed hardwood forest. The micro-morphologic data revealed small, rather rounded isodiametric spores averaging ca. $7\ \mu\text{m}$; pileipellis hyphae arranged in an ixocutis; abundant clamp-connections; and broad inflated hyphae in the subpellis and hymenophoral trama. All these data supported it as a species of *Entoloma* sect. *Entoloma* (sensu Noordeloos 1981, 1992, 2004). From a macro-morphological point of view, the basidiomes recalled the *E. prunuloides* group but differed from those of a typical *E. prunuloides* not only in pileus colour—sepia ochraceous rather than whitish—but also for their slender aspect (quite unlike the stout and fleshy basidiomes of *E. prunuloides*; Noordeloos 1992, 2004). This collection was therefore assigned to *E. prunuloides* var. *obscurum*, a variety described in 2004 and later raised to species rank and renamed as *E. ochreoprunuloides* (Morgado et al. 2013).

The morphologically closest species to *E. ochreoprunuloides* is *E. luteobasis* Ebert & E. Ludw. (described from Germany and Italy), which differs mainly by its slightly to distinctly yellow or ochraceous stipe base (Ebert et al. 1992, Noordeloos & Hausknecht 1998, Noordeloos 2004). Because the holotype of *E. luteobasis* is missing from the Leiden herbarium (L; Nicolien Sol, pers. comm.), we turned to an Italian collection of *E. luteobasis* (WU 17842) described in Noordeloos & Hausknecht (1998) and Noordeloos (2004). Our ITS sequence analyses revealed that this collection is conspecific with *E. ochreoprunuloides*. This suggests that *E. luteobasis* (proposed in 1992) might be considered an earlier name for *E. ochreoprunuloides* (published in 2013). However, additional collections and sequences of both taxa and further phylogenetic analyses will be required to test this potential synonymy.

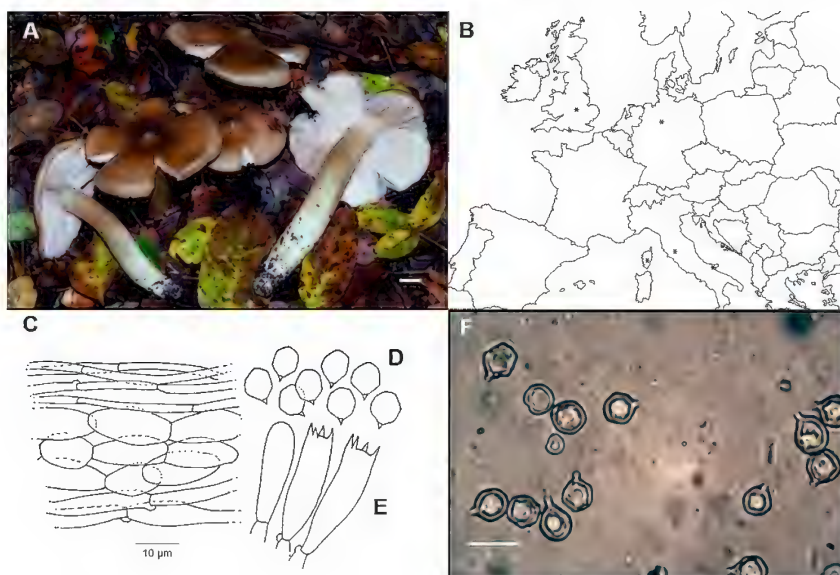


FIG. 1. *Entoloma ochreoprunuloides* (TO 3327). A. Habit. B. Map of *E. ochreoprunuloides* distribution. C. Pileipellis. D. Spores. E. Basidia. E. Spores (in water). Scale bars: A = 1 cm; C–F = 10 µm.

From a morphological point of view, delimiting species in the *E. prunuloides-bloxamii* group is no easy task. Morgado et al. (2013) published a box plot of spore dimensions for all the species of the group that showed *E. ochreoprunuloides* spores as among the smallest in the whole section. However, our Tuscan collection falls within the limits of spore size and shape for the species ($5.9\text{--}7.1 \times 5.7\text{--}7.2$ µm, $Q = 1.0\text{--}1.16$, $Q_{av} = 1.04$; Morgado et al. 2013). Our data also agree well with the WU 17842 (*E. luteobasis*) figure and spore dimensions in Noordeloos & Hausknecht (1998; $6.6\text{--}7.6 \times 6.2\text{--}7.5$ µm, av. 7.0×6.6 µm, $Q_{av} = 1.05$). Spore dimensions can be used to distinguish *E. ochreoprunuloides* from *E. prunuloides* (with a spore average ca. 1 µm larger; Morgado et al. 2013). Typically, *E. prunuloides* tends to be more robust and to have pale (whitish to light ochraceous) tinges on the pileus (Noordeloos 1992, 2004), but it should be stressed that pileus colours are rather variable and should be used only in connection with other characters. In contrast, our collection showed sepia brown ochraceous colours, which are probably the most typical for the species; Noordeloos & Hausknecht (1998) reported a brown (from chocolate brown through orange brown) pileus with a greyish

margin. Morgado et al. (2013) also described a new form (*E. ochreoprunuloides* f. *hyacinthinum*) with violet or pink tinges on both pileus and stipe. DNA sequences from two collections of this form, however, show an almost complete identity with the typical form (Morgado et al. 2013).

With its dark pileus colors and small spores, the Canadian species *E. pseudoprunuloides* is morphologically closer to *E. ochreoprunuloides* than to *E. prunuloides*. However, the phylogenetic analysis by Morgado et al. (2013) placed it as sister to *E. prunuloides*.

Our report widens the distribution of *E. ochreoprunuloides* to include Italy, in addition to Germany (NordRhein-Westfalen, Ibbenbüren, holotype), UK, and France (Corsica). A map with all of the localities is presented in FIG. 1B.

Acknowledgments

The authors are grateful to Anton Hausknecht (Maissau, Austria) for giving us a collection of *Entoloma luteobasis* and to Nicolien Sol (Naturalis Biodiversity Center, Leiden, The Netherlands) for providing information on the holotype collection of *E. luteobasis*. Thanks are due also to Gabriel Moreno (Univ. Alcalá de Henares, Madrid, Spain) and Tim Baroni (State University of New York – College at Cortland, New York) for reviewing the manuscript and providing useful suggestions.

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***Phaeocollybia pakistanica* sp. nov., the first representative of the genus from Pakistan**

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ABSTRACT—A new species of *Phaeocollybia*, *P. pakistanica*, is described that represents the first record of the genus from Pakistan. Diagnostic characters include purplish red to brownish red pilei, lilaceous gills, a monopodial radicating cartilaginous stipe, small ellipsoidal verruculose brown spores, and thin-walled subcapitate cheilocystidia. ITS-nrDNA sequence analyses support *P. pakistanica* as an independent new species.

KEY WORDS—Agaricomycetes, *Hymenogastraceae*, coniferous forest, pseudorhiza, Swat district

Introduction

Phaeocollybia R. Heim is a genus of agaricoid fungi, currently accepted in *Hymenogastraceae* (Matheny et al. 2006). The genus is easy to recognize in the field due to the presence of the cartilaginous stipe and deeply rooting pseudorhiza, moist to viscid umbonate pilei, and brown spores. Microscopically the genus is characterized by gelatinous tissues, roughened brown spores with an apical callus, the presence of cheilocystidia, and tibiiform diverticula (Norvell & Exeter 2008). The genus is widely distributed in forested temperate regions worldwide and currently comprises about 95 described species (<http://www.indexfungorum.org/>).

Species later placed in *Phaeocollybia* were first included by Fries (1838) in the heterogeneous assemblage of brown-spored agarics within the [unranked; section] *Gymnoti* under [unranked; subgenus] *Naucoria*. Kummer (1871) and Quélet (1872) independently raised *Naucoria* to generic level. Heim (1931)

established *Phaeocollybia* with *P. lugubris* (Fr.) R. Heim as type to accommodate *Naucoria* species characterized by yellow brown spores, viscid caps, and cartilaginous radiating stipes.

The genus is widely distributed throughout forests in Great Britain, Europe, Asia, Australasia, and South, Central, and North America (Norvell 1998a). Nowhere common, the genus can be considered quite rare for Pakistan, and there is no previous *Phaeocollybia* record for the country (Ahmad et al. 1997). In this paper we report the first occurrence of *Phaeocollybia* for Pakistan and describe a new species based on morphological and molecular analyses.

The specimens were collected from moist temperate forests of Malam Jabba, Khyber Pakhtunkhwa, Pakistan. Malam Jabba valley, an important biodiversity hotspot for Pakistan that hosts a variety of important medicinal and aromatic plants (Sher & Al-Yemeni 2011), lies in northern Pakistan between the Himalayas and the Hindu Kush foothills. The region (35°20'–35°45'N 72°12'–73°32'E) lies 52 km from Saidu Sharif, the capital of Swat District in the Malakand division of Khyber Pakhtunkhwa, bordering Shangla District to the northeast and District Buner to the southwest. Altitudes range from 990 m (valley entrance) to 2880 m (the highest peak of Shagar Sar; Ali et al. 2011). The valley benefits from varied agro-climatic conditions and geographic areas that facilitate a large number of important plants (Ali & Qasier 1986). The lush green coniferous forests of the Malam Jabba are also home to large numbers of important herbs, and the forest floors rich in humus and moist environments favor the growth of many macrofungi.

Materials & methods

Collection, morphological and anatomical study of basidiomata

Basidiomes were collected according to Lodge et al. (2004) slightly modified by excavation to trace their pseudorhizal origins (Norvell 1998b). Fresh specimens were photographed and important ephemeral characters and habitat data were recorded in the field. Surface features were examined using a hand lens. The collected specimens were covered in blotting paper prior to transport to the laboratory where they were dried at 40–50°C using a fan heater.

Macroscopic descriptions are based on the fresh field collections. Colors were coded according to Munsell (1975). Certain morphological and developmental terms (e.g., tibiiform diverticula, vertical-monopodia, pseudorhizae) follow Norvell (1998a,b). The dried specimens have been deposited in Swat University herbarium (SWAT) and Herbarium, University of the Punjab, Pakistan (LAH).

For microscopic examination, tissues were rehydrated in distilled water and mounted in 5% KOH, with Congo red as a stain. Anatomical features were measured using calibrated Piximètre software connected to a BOECO BM 120 compound microscope through a Byomic MVV 3000 microscopic camera and visualized on a computer screen. Twenty basidiospores, basidia, and cystidia were measured; Q = the length/width ratio

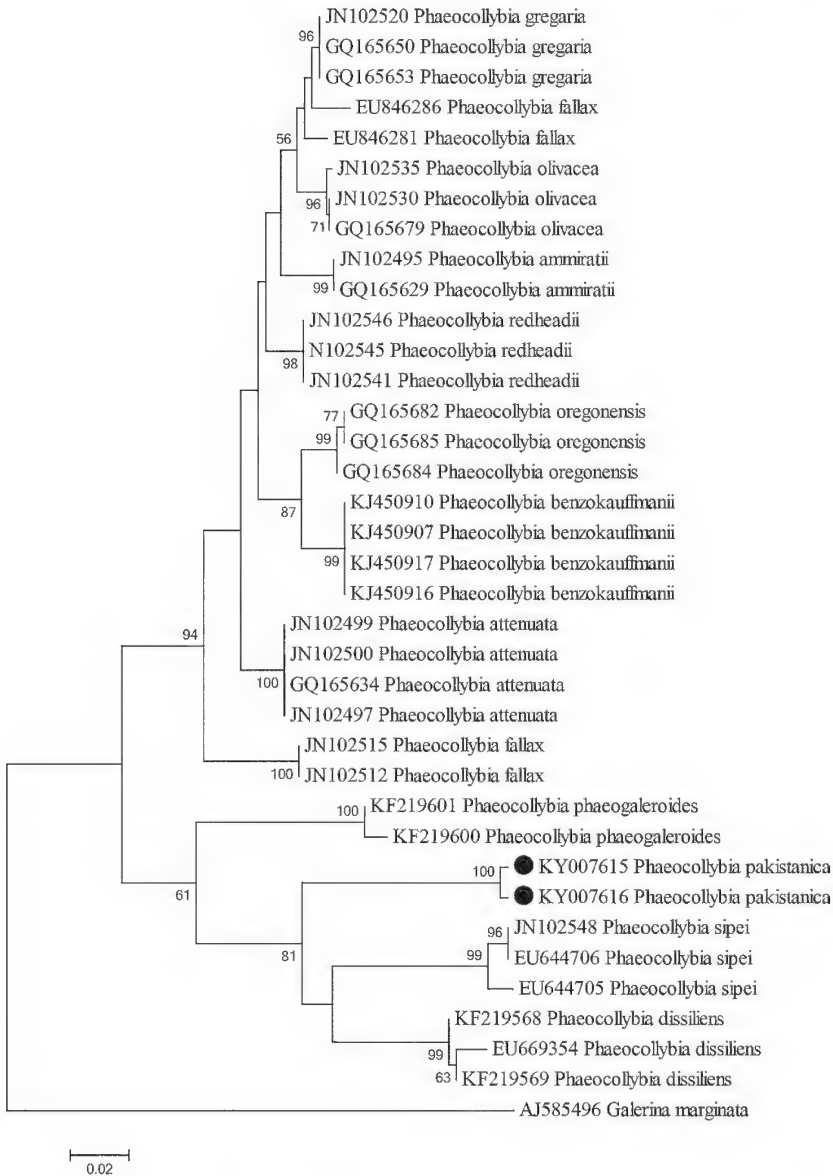


FIG. 1. Maximum Likelihood phylogeny of *Phaeocollybia* spp. Cluster support percentage is shown next to the branches. The initial tree for the heuristic search was obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated by the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths representing number of substitutions per site. The analysis involved 34 nucleotide sequences. There were a total of 422 positions in the final dataset.

of a single spore; Q_e = average length/width ratio of all spores; Me = average $L \times W$ of all spores measured.

DNA extraction, amplification, sequencing, and molecular phylogenetic analysis

About 50 mg of dried gills were ground into fine powder using liquid nitrogen, and the DNA was extracted according to Porebski et al. (1997). The universal primer pair ITS1F and ITS 4 (White et al. 1990, Gardes & Bruns 1993) was used to amplify the rDNA ITS (ITS1+5.8s+ITS2) region in 50 μ L reaction volumes following Gardes & Bruns (1993). The PCR amplified product was sequenced by Beijing Genomic Institute (Hong Kong). BLAST analysis was performed using the National Center for Biotechnology Information (USA) database, and closely matching sequences were downloaded for further phylogenetic analysis. Sequences were aligned using MEGA 6 software (Tamura et al. 2013). *Galerina marginata* (Batsch) Kühner was selected as outgroup. Sequences are deposited in GenBank.

Results

Phylogenetic analysis

FIG. 1

Amplification of the entire ITS region using the ITS1 and ITS4 primers generated a 690bp sequence. The initial BLAST comparison of the Pakistan sequences showed 85% identity and 96% query cover with *Phaeocollybia dissiliens* A.H. Sm. & Trappe (KF219569, KF219568) and 84% identity and 96% query cover with *P. sipei* A.H. Sm. (JN102548, EU644705, EU644706). The MEGA6 maximum likelihood analysis clustered our Pakistan sequences with the *P. dissiliens* and *P. sipei* sequences in a separate clade with a strong bootstrap value of 81%, thereby supporting *P. pakistanica* phylogenetically as an independent species.

Taxonomy

***Phaeocollybia pakistanica* J. Khan, Sher & Khalid, sp. nov.**

FIGS 2, 3

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Differs from *Phaeocollybia sipei* by possession of clamp connections, and from both *P. dissiliens* and *P. sipei* by its lilaceous cap and gill color.

TYPE: Pakistan, Khyber Pakhtunkhwa Province, Swat District, Malam Jabba, 2500 m a.s.l, small groups on forest soil near *Abies pindrow* (Royle ex D. Don) Royle and *Pinus wallichiana* A.B. Jacks., 14 Sep. 2015, Junaid Khan MJ1560 (Holotype, SWAT15-1560; GenBank KY007615)

ETYMOLOGY: referring to Pakistan, the country where the species was first collected.

PILEUS 70–90 mm diam., conical to cuspidate when young, becoming convex and papillate at maturity, margin initially straight, later uplifted to revolute and undulating; edge and outer margin striate to rugulose almost two-thirds toward the central disc; viscid to glutinous when wet, shiny when dry; hygrophanous with a darker edge, color initially purplish red (10RP6), dulling to brownish red (10R7/4) and finally brownish (10R6/2) on exposure. CONTEXT concolorous with or slightly paler than pileus, thin, ≤ 3 mm thick at the disc and 1 mm at the



FIG. 2. *Phaeocollybia pakistanica* (holotype, SWAT15-1560). A. basidiomata in situ; B. detail of shiny pileus and violet lamellae; C. purplish-red stipe and lamellae; D. excavated basidioma showing purplish stipe apex. Scale bars = 10 mm.

outer edge, moist to dry, soft; odor fruity to mildly mushroomy; taste mild, not distinctive. LAMELLAE adnexed to slightly sinuate, close, color purplish with a reddish tone (7.5 RP7) at least when young before turning brown (10R4/8), edges eroded, more or less straight, crisped or curled toward the stipe in some specimens; lamellulae frequent, with crisped terminal ends, $L+l/cm \leq 25$. STIPE including pseudorhiza overall ≤ 160 mm, aerial portion < 100 mm long, apex 5 mm diam.; central, terete, cylindrical or slightly widening towards the base, even slightly bulbous in some specimens; concolorous with the cap or slightly paler, with a paler purplish tinted (7.5 R8/2) upper portion and darker (7.5R6/8) lower portion without any purplish tints, glabrous or slightly fibrous when young, not bruising, hollow, cartilaginous, cortex 1-1.5 mm thick, lined with fibrils. PSEUDORHIZA ≤ 60 mm, (approximately 1/3 of overall stipe+pseudorhiza length), vertical monopodial, tapering with a blunt origin, more or less concolorous with the aerial stipe, but slightly paler orange in upper part and slightly darker below.

BASIDIOSPORES (6-)6.4-7.1(-7.2) $\times$ (3.4-)3.6-4.5 μm , $Q = (1.3)$ 1.5-1.8 (1.9), $Me = 6.7 \times 4.1 \mu m$, $Q_e = 1.6$, ellipsoid to elongate in side view, ovoid in face view, finely verruculose in oil immersion, apiculate, brownish amber in KOH. BASIDIA $\leq 30 \mu m$ long, narrowly clavate to cylindrical, hyaline, 4-spored,

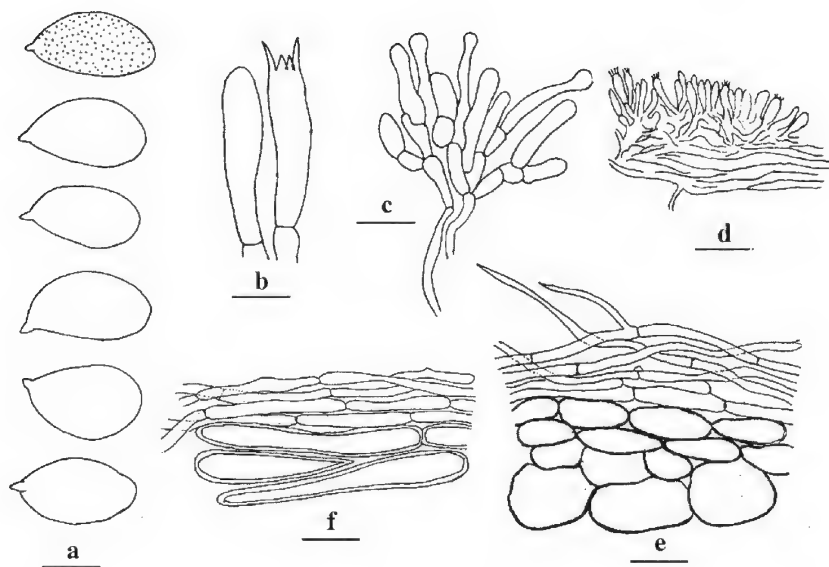


FIG. 3. *Phaeocollybia pakistanica* (holotype, SWAT15-1560). A. basidiospores; B. basidiole and basidium; C. subcapitate cheilocystidia; D. hymenium showing immature and sterigmate basidia; E. pileipellis with narrow, branched and anastomosing hyphae of the suprapellis (top) overlying shorter and wider unbranched subpellis hyphae (middle) and wider thick-walled tramal hyphae (bottom); F. pseudorhiza with rhizopellis layer shown above thick-walled tramal cells. Scale bars: A = 4.5 μm ; B = 12 μm ; C = 20.5 μm ; D = 10 μm ; E = 21 μm ; F = 35 μm .

sterigmata $\leq 3 \mu\text{m}$ long. CHEILOCYSTIDIA $< 40 \mu\text{m}$ long, sub-capitate, numerous, thin-walled. PILEIPELLIS bilaminar, suprapellis a $\leq 23 \mu\text{m}$ -thick layer with narrow ($\leq 2\text{--}3 \mu\text{m}$ diam) branched, gelatinized, pale brownish to hyaline hyphae with occasional clamp connections at the septa overlying a slightly darker (KOH) subpellis composed of somewhat inflated thick-walled cells ($\leq 10 \mu\text{m}$ diam and lacking clamp connections) interspersed with $\leq 10 \mu\text{m}$ diam, non-septate, golden brown, branched oleifers. PILEUS TRAMA cells shorter, $\leq 25 \mu\text{m}$ diam., thick-walled. STIPITPELLIS cells $\leq 100 \times 10 \mu\text{m}$, cylindrical, thick-walled. PSEUDORHIZAL PELLIS cells narrow, $\leq 5 \mu\text{m}$ diam, elongate, without any clamps. STIPE & PSEUDORHIZA TRAMAS sarcodimitic, with thinner branching flexuous cells intermixed among 'vessel elements' that are thick-walled, $\leq 250 \times 30 \mu\text{m}$, more or less fusoid, brown to golden in KOH. CLAMP CONNECTIONS very rare; observed occasionally in pileipellis. TIBIFORM DIVERTICULA present on pseudorhizal pellis.

ADDITIONAL SPECIMENS EXAMINED: PAKISTAN, KHYBER PAKHTUNKHWA PROVINCE, SWAT DISTRICT, Malam Jabba, 2500 m asl, in small groups under *Abies pindrow*, 10 Aug

2014, Junaid Khan MJ65 (LAH35009); 31 Jul, 2016, Junaid Khan MJ1660 (SWAT16-0021)

Discussion

Molecular analysis clusters the present Pakistani collection with *P. dissiliens* and *P. sipei*, but *P. pakistanica* is both morphologically and molecularly well separated from those two species. *Phaeocollybia dissiliens* (Smith & Trappe 1972) and *P. sipei* (Smith 1957), both originally described from the North American Pacific coast, are distinguished by their bright orange to brownish orange coloration and lack the lilaceous gill colors characterizing *P. pakistanica*. The vertical monopodial pseudorhiza of *P. pakistanica* lacks the thread-like to racemose pseudorhiza of *P. dissiliens* and *P. sipei* noted by Norvell & Exeter (2008). The finely verruculose or punctate roughened ellipsoidal basidiospores are also morphologically similar to the two North American species. The rarity of clamp connections in the Pakistani collections (with a few found only in the suprapellis) further distinguishes *P. pakistanica* from *P. dissiliens* (with clamp connections relatively frequent in the pileus suprapellis and at the cheilocystidial and basidial bases) and *P. sipei* (which lacks clamp connections throughout).

Phaeocollybia spoliata E. Horak, described from India, slightly resembles *P. pakistanica* in its reddish coloration (although orange-brown more than purplish) and striate cap margin but is easily distinguished by its larger ($9.5\text{--}10 \times 5\text{--}5.5\text{ }\mu\text{m}$) more heavily ornamented almond-shaped spores (Horak 1974). *Phaeocollybia rancida* E. Horak, also described from high coniferous forests in India and characterized by lilaceous violet lamellae, capitate cheilocystidia, and ellipsoid basidiospores, is easily separated by its ochraceous coloration, glutinous pilei, slightly smaller ($5\text{--}6 \times 3\text{--}3.5\text{ }\mu\text{m}$) basidiospores, and absence of clamp connections (Horak 1974).

Acknowledgements

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***Mucor indicus* isolated from the semiarid region of Brazil: a first record for South America**

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ABSTRACT—A specimen of *Mucor indicus* was isolated from a dung sample collected in the city of Sertânia in the Brazilian state of Pernambuco and its identity confirmed through LSU rDNA sequence analysis. The taxon is described and illustrated for the first time from South America. An identification key is provided for *Mucor* species from the northeast Brazilian semiarid region (Caatinga).

KEYWORDS—lower fungi, phylogenetic analysis, *Mucoromycotina*, *Zygomycota*

Introduction

Mucor Fresen. (*Mucoraceae*) comprises species characterized by the formation of non-apophysate sporangia, producing simple or branched sporangiophores that emerge directly from the substrate. Few species have rhizoids and stolons are not produced (Benny 2014). This genus has a worldwide distribution and most of the species described are saprobes commonly isolated from a wide variety of substrates such as soil, stored grains, fruits, rotting vegetables, fleshy agarics, and herbivore dung (Viriato & Trufem 1985, Alves et al. 2002, Schoenlein-Crusius et al. 2006, Jacobs & Botha 2008, Santiago & Souza-Motta 2008, Santiago et al. 2013).

Although over 300 species have been cited in the literature (Jacobs & Botha 2008), the exact number of authentic taxa is unknown. According to Gherbawy et al. (2010), it is possible that the number of authentic species could range

between 50 and 75. Schipper (1973, 1975, 1976, 1978) described 39 species, four varieties, and 11 forms of *Mucor*. Subsequently, 17 additional species have been proposed (Mehrotra & Mehrotra 1978, Mirza et al. 1979, Subrahmanyam 1983, Chen & Zheng 1986, Schipper 1989, Schipper & Samson 1994, Watanabe 1994, Zalar et al. 1997, Pei 2000, Alves et al. 2002, Jacobs & Botha 2008, Álvarez et al. 2011, Hermet et al. 2012, Madden et al. 2012).

Previous studies have treated *Mucor* as polyphyletic (O'Donnell et al. 2001, Kwasna et al. 2006, Jacobs & Botha 2008, Budziszewska & Piatkowska 2010, Álvarez et al. 2011). Based on the phylogeny of ITS and LSU rDNA regions of several mucoralean species, Walther et al. (2013) observed that some *Mucor* species with curved sporangiophores represent a natural grouping with *Backusella* Hesselt. & J.J. Ellis, and transferred nine *Mucor* species to *Backusella*.

The aim of the present study was to describe and illustrate *M. indicus* from dung samples collected in the city of Sertânia, Pernambuco, Brazil. An identification key for *Mucor* species from the semiarid region of Brazil is also provided.

Material & methods

Isolation and morphological & physiological identification of *M. indicus*

Samples of goat (*Capra hircus* Linnaeus) dung were collected at the Agronomic Institute of Pernambuco stations, located in the city of Sertânia (8°03'38"S 37°13'32"W) in the state of Pernambuco, 326 km away from Recife, Brazil. The mean annual temperature is 25°C, with rainfall of 635 mm per year. The area has a hyperxerophilic Caatinga vegetation. The samples were placed in clean plastic bags for transportation to the laboratory and then incubated in triplicate in moist chambers for 10 days. The plates were left on a bench at room temperature (28 ± 2°C) exposed to alternating light and dark periods. Fragments of mycelium were removed directly from Petri dishes under a Leica EZ4 stereomicroscope and transferred to Petri dishes with malt extract agar (MEA) (Benny 2008). The specimen was identified through observation of macroscopic (color, appearance, and diameter of the colony) and microscopic (e.g., shape and size of sporangiophores, columellae, sporangia, and sporangiospores) characteristics based on the descriptions of Schipper (1978). The thermotolerance of *M. indicus* was confirmed by growing it at 40°C for 7 days. The colony colors were designated according to Maerz & Paul (1950).

Molecular analysis

Cultures grown in test tubes containing malt extract were incubated at 28°C for 6 days to generate fungal biomass. The material was transferred to 2 mL microtubes with screw caps. Subsequently, 0.5 g acid-washed glass beads of two different diameters (150–212 µm and 425–600 µm, 1:1; Sigma, USA) were added to each tube. The material was crushed by stirring at high speed in a FastPrep homogenizer. The genomic DNA extraction procedure was conducted as described by Góes-Neto et al. (2005). The mycelium was washed with chloroform: isoamyl alcohol (24:1) and then homogenized

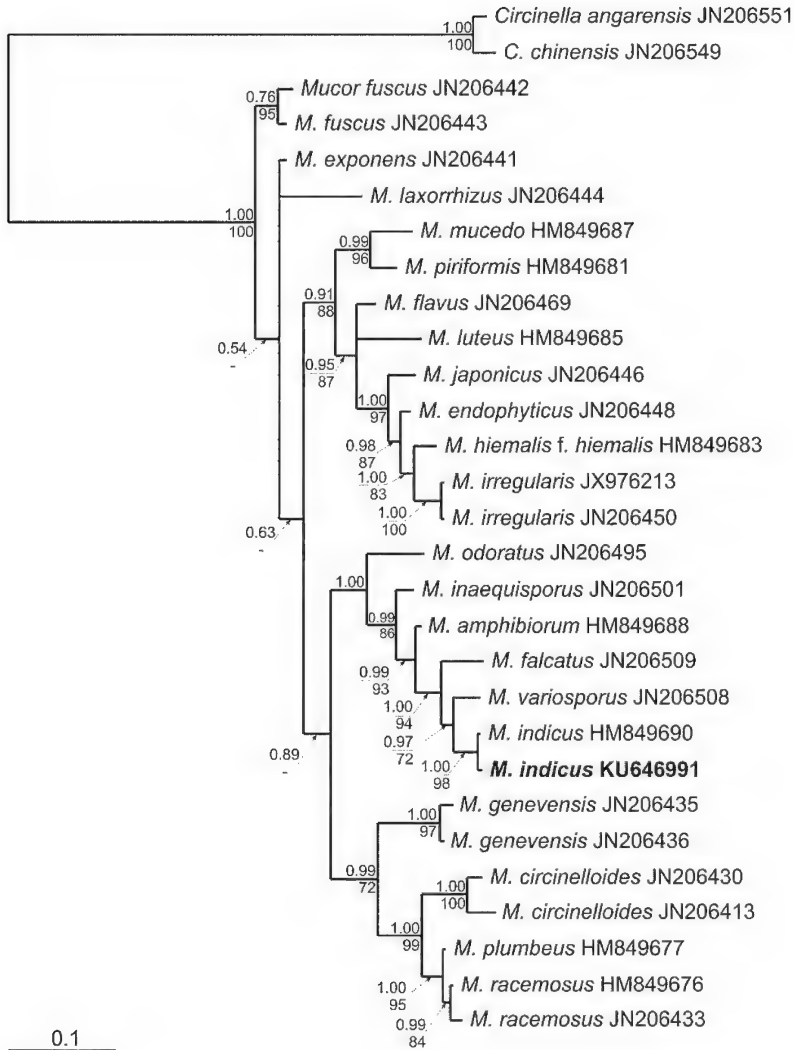


Fig. 1. Phylogenetic tree of *Mucor* species constructed using partial LSU rDNA sequences. *Circinella angarensis* and *C. chinensis* were used as outgroup. Sequences are labeled with their GenBank accession numbers. Branch support values are from Bayesian inference (above) and maximum likelihood (below) analyses. The sequence obtained in this study is in boldface.

in 2% cetyltrimethylammonium bromide (CTAB) buffer. The DNA was precipitated in chilled isopropanol, washed with 70% ethanol, and resuspended in 50 µL ultrapure water.

The primer pairs LR1/LSU2 were used to amplify a fragment of the large subunit (LSU) nuclear ribosomal DNA (rDNA) (van Tuinen et al. 1998, Santiago et al. 2014). The polymerase chain reactions were carried out as described by Oliveira et al. (2014). The newly obtained sequence was deposited in the National Center for Biotechnology Information GenBank database (accession number KU646991).

Phylogenetic reconstructions were obtained by analyzing the partial LSU rDNA sequences. The fungal sequences were aligned with ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999). Prior to the phylogenetic analysis, the optimal nucleotide substitution model was estimated using Topali 2.5 (Milne et al. 2004). Bayesian inference (two runs over 10^6 generations with a burn-in of 2500) analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), launched from Topali 2.5.

Results

Phylogenetic analysis

The Blastn analysis of the LSU sequences showed a 99% similarity between our Brazilian material (URM7222) and *M. indicus* (CBS 226.29; GenBank HM849690). Phylogenetic analysis of the sequence datasets (FIG. 1) showed that URM7222 formed a well-supported clade (BI 1.00/ML 98%) with the sequence of *M. indicus* (HM849690).

Taxonomy

Mucor indicus Lendn., Bull. Soc. Bot. Genève 21: 258 (1930).

FIG. 2

COLONY initially white and becoming deep yellowish (MP 11C1 – Amber White) with yellowish reverse (MP 11J6), reaching 9.4 cm in diam. and 9 mm in height after 4 days in MEA at 28°C. SPORANGIOPHORES erect (rarely circinate), repeatedly sympodially branched, with long branches, (4–)5–12.5(–14.5) µm diam., hyaline to yellowish, smooth-walled. SPORANGIA globose to subglobose, (22.5–)32.5–45(–50) µm diam., brownish, with diffuent wall. COLUMELLAE globose (27.5–)30–47.5(–50) µm, subglobose (25.5–)27.5–28.5 × 42.5–45(–47.5), or applanate (18–)20–21.25 × 27.5–30(–32.5) diam., grayish or yellowish brown, smooth-walled; COLLARS small and infrequent. SPORANGIOSPORES hyaline, smooth-walled, often ellipsoid (3.75–)4–6.25 × 3–6.5(–7.5) µm and subglobose 4–6(–7.5) µm in diam. CHLAMYDOSPORES present. ZYGOSPOANGIA not observed. Growth reasonably good at 40°C (7.6 cm in 168 hours) in MEA with rare sporangioophore production and poor sporulation.

HABITAT: Recorded on goat dung in the semiarid region of Pernambuco, northeastern Brazil.

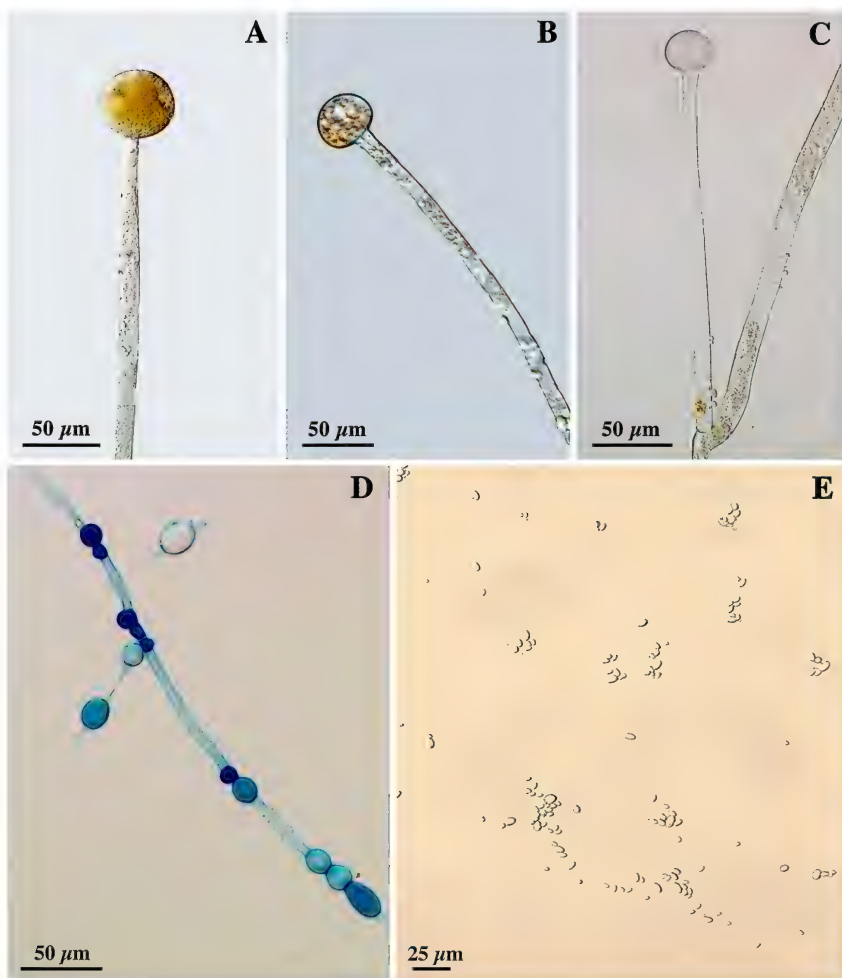


Fig. 2. *Mucor indicus* (URM 7222): A. Sporangiophore simple with sporangia; B. Sporangiophore simple with columella; C. Sporangiophore branch with columella; D. Chlamydospores; E. Sporangiospores.

SPECIMEN EXAMINED: BRAZIL, PERNAMBUCO, Sertânia: Instituto Agronômico de Pernambuco (IPA), 8°03'38"S 37°13'32"W, from goat dung, 5.XI.2014, leg. C.A.F de Souza (URM 7222).

GEOGRAPHIC DISTRIBUTION: India (Lendner 1930, Abe et al. 2004), Poland (Szwedek-Trzaska & Glowacka 2011), Spain (Luna et al. 1986, García-Pantaleón et al. 1992, Herrero et al. 1996), USA (Oliver et al. 1996, Sobel 2001, Álvarez et

al. 2009), and Vietnam (Lee & Fujio 1999). Our Brazilian collection represents the first report from South America.

Discussion

The morphological characteristics of *M. indicus* reported here show a close similarity with the description by Schipper (1978), although there are differences in the diameter of sporangiospores and columellae. Sporangiospore sizes reported in the literature ($5.4\text{--}5.7 \times 4.4\text{ }\mu\text{m}$; Schipper 1978) are smaller than our maximum measurements, whereas columellae were larger ($\leq 56 \times 53\text{ }\mu\text{m}$; Schipper 1978) than observed in our isolate. *Mucor indicus* strains may exhibit morphological similarities to *M. circinelloides* Tiegh. (Schipper 1978). However, *M. indicus* lacks the abundant short branches reported for *M. circinelloides* and its predominantly globose columellae differ from the obovoid type found in *M. circinelloides*. Moreover, the thermotolerance inherent to *M. indicus* is not found in *M. circinelloides* (Schipper 1978). Walther et al. (2013) have reported that although some *M. indicus* cultures morphologically resemble *M. circinelloides*, there are clear genetic differences between these two species.

The LSU phylogenetic tree (Fig. 1) suggests that *M. indicus* and *M. variosporus* Schipper are genetically closely related. Morphologically, however, *M. indicus* colonies are lower than those of *M. variosporus*, which can reach 20 mm in height (Schipper 1978). Differences in sporangiophore diameters have also been observed, with *M. variosporus* producing larger ($14\text{--}23\text{ }\mu\text{m}$) sporangiophores. Finally, *M. variosporus* sporangia are yellow and larger ($\leq 150\text{ }\mu\text{m}$ diam.) than those of *M. indicus*, which are brownish and smaller.

This manuscript reports the first occurrence of *M. indicus* in South America, thereby expanding our knowledge of mucoralean distribution. In the semiarid region of Brazil (Caatinga), other *Mucor* species have been isolated, such as *M. circinelloides* Tiegh. f. *circinelloides*, *M. hiemalis* Wehmer, *M. luteus* Linnem. ex Wrzosek., *M. prayagensis* B.S. Mehrotra & Nand ex Schipper, *M. racemosus* Fresen., and *M. subtilissimus* Oudem. (Santiago 2016). During a recent survey of *Mucorales* from other Brazilian semiarid regions, *M. circinelloides* Tiegh. f. *griseocyanus* (Hagem) Schipper, *M. circinelloides* Tiegh. f. *janssenii* (Lendn.) Schipper, *M. circinelloides* Tiegh. f. *lusitanicus* (Bruderl.) Schipper, *M. racemosus* Fresen. f. *racemosus*, and *M. variosporus* Schipper were also isolated (unpublished data).

Identification key for *Mucor* taxa isolated from the semiarid region of Brazil

- 1. Sporangiospores regular in form and size 2
- 1. Sporangiospores variable in size and shape: globose, ovoid, cylindrical, and fusiform *M. variosporus*

2. Sporangiophores unbranched or slightly sympodially branched 3
2. Sporangiophores repeatedly branched 6
3. Columellae globose to subglobose or globose to applanate;
 sporangiospores $\leq 15 \mu\text{m}$ long with or without a granule 4
3. Columellae spherical or ellipsoidal with a truncated base;
 sporangiospores ≤ 8 or $10 \mu\text{m}$ long 5
4. Sporangiophores unbranched; columellae subglobose to applanate;
 sporangiospores ellipsoidal or flattened on one side *M. prayagensis*
4. Sporangiophores sparsely branched; columellae globose to subglobose
 (rarely ellipsoidal); sporangiospores ellipsoidal with a granule at
 each end *M. subtilissimus*
5. Columellae ellipsoidal with a truncate base; sporangiospores ellipsoid,
 sometimes flattened on one side, $2.5\text{--}10 \times 2\text{--}7.5 \mu\text{m}$ *M. hiemalis*
5. Columellae globose;
 sporangiospores long-elliptical and fusiform $2.5\text{--}8.1 \times 1\text{--}5 \mu\text{m}$ *M. luteus*
6. Absence of growth at 40°C 7
6. Good growth at 40°C *M. indicus*
7. Sporangiophores sympodially branched; chlamydospores present or not,
 when present not abundant and never formed in sporangiophores or
 columellae 8
7. Sporangiophores sympodially and monopodially branched;
 chlamydospores abundant, some formed in sporangiophores and
 columellae *M. racemosus* f. *racemosus*
8. Colonies initially white, then becoming gray;
 sporangiospores $\leq 10 \mu\text{m}$ in diam.; sporangia brownish-black 9
8. Colonies initially yellow than turning brownish;
 sporangiospores $\leq 17 \mu\text{m}$ in diam.; sporangia gray-brownish 10
9. Sporangiospores ellipsoid *M. circinelloides* f. *griseocyanus*
9. Sporangiospores globose to slightly subglobose *M. circinelloides* f. *janssenii*
10. Columellae obovoid;
 sporangiospores ellipsoid *M. circinelloides* f. *circinelloides*
10. Columellae globose;
 sporangiospores ellipsoid, some irregular in shape *M. circinelloides* f. *lusitanicus*

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***Blodgettia saprophytica* sp. nov. and *Uberispora tropicalis*, new records from southern China**

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ABSTRACT—A new species, *Blodgettia saprophytica*, is described and illustrated from specimens collected on dead branches in Guangxi Province, China. *Uberispora tropicalis* is newly recorded from China.

KEY WORDS—hyphomycetes, taxonomy

Introduction

Blodgettia Harv. (Harvey 1858), comprising algal and fungal elements, has commonly been considered an illegitimate name under the former ICBN Art. 70 (Stafleu et al. 1972) so that *Blodgettia* E.P. Wright (Wright 1881) has been used for the fungal element. However, Seifert et al. (2011), who interpreted Wright's treatment as emending *Blodgettia* Harv. by selecting the fungal element, as now required by the current International Code of Nomenclature (McNeill et al. 2012), regarded *Blodgettia bornetii* E.P. Wright as a later synonym of *B. confervoides*. Two other species, *B. indica* Subram. and *B. aquatica* Udaiyan, have been included in the genus (Subramanian 1954, Udaiyan 1992).

Here we propose an additional *Blodgettia* species on dead branches from southern China. We also report *Uberispora tropicalis* on dead branches as a new record from China.

Material & methods

Samples were processed, examined, and photographed following the methods described in Xia et al. (2014). Adobe Photoshop 7.0 was used to process individual

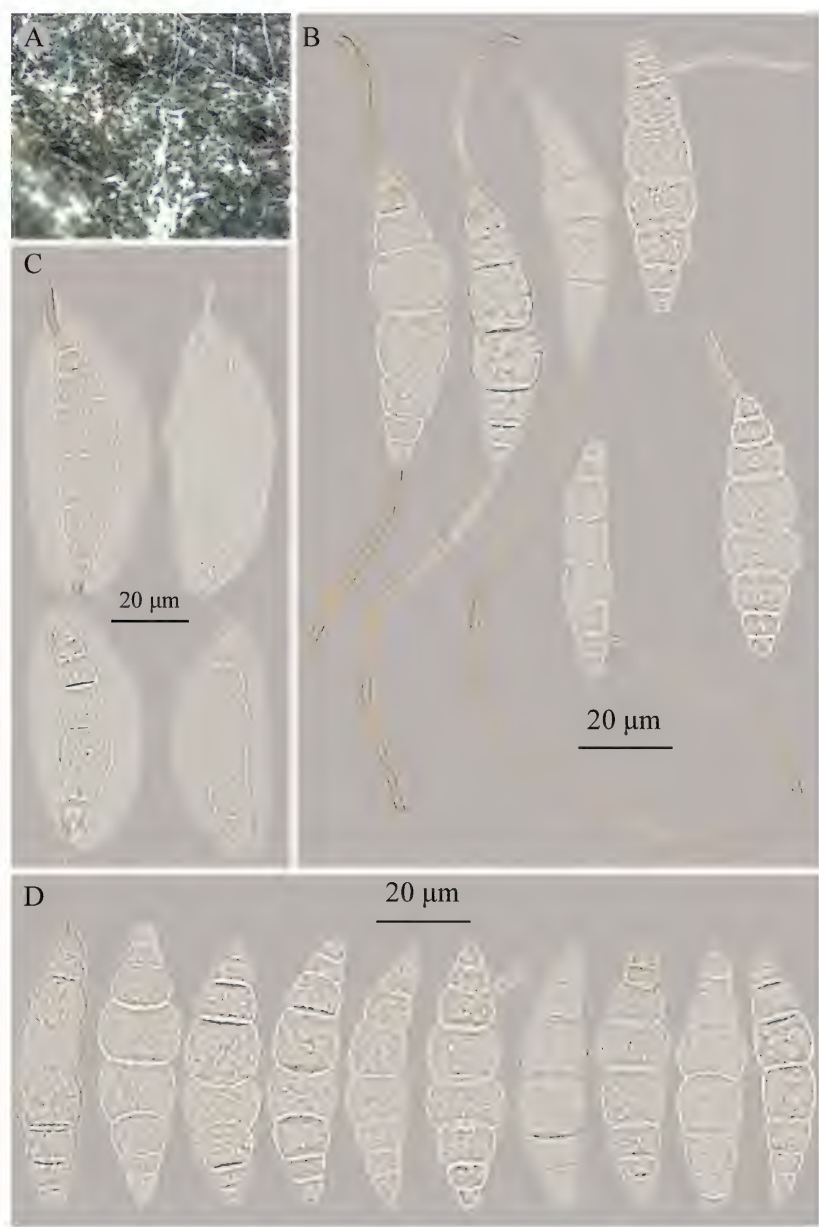


FIG. 1. *Blodgettia saprophytica* (holotype, HSAUP H7855). A. Colonies on natural substratum. B. Conidia and hyphae. C. Conidia covered by a mucilaginous tunica. D. Conidia.

images into photomicrographic plates, with backgrounds modified for esthetic reasons. In view of our failure to obtain cultures of this species, no molecular data are presented here. The specimens are deposited in the Herbarium of Department of Plant Pathology, Shandong Agricultural University, Taian, Shandong, China (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS).

Blodgettia saprophytica C.L. Yang, Z. Li & X.G. Zhang, sp. nov.

FIG. 1

MYCOBANK MB 819496

Differs from *Blodgettia aquatica* and *B. indica* by its larger, hyaline or subhyaline conidia with more numerous septa and their occasional coverage by a mucilaginous tunica.

TYPE: China, Guangxi Province, Nanning, Longhushan, on dead stems of unidentified broadleaf tree, 15 Nov. 2015, C.L. Yang (**Holotype**, HSAUP H7855; **isotype**, HMAS 245638).

ETYMOLOGY: In reference to the saprophytic habit on dead wood.

COLONIES on the natural substratum effuse, inosculating, hyaline or subhyaline, twinkling. Mycelium superficial and immersed, composed of very long and yellowish, mostly unbranched, aseptate, smooth hyphae. CONIDIA solitary, broadly fusiform, straight or slightly curved, moniliform, sometimes covered by a mucilaginous tunica, forming strings of eight cells, 7-septate, distinctly constricted at the septa, with the 2 middle cells greatly enlarged, and guttulate in every cell, globose or ellipsoidal, smooth, hyaline or subhyaline, the two apical cells attenuated and sometimes triangular, with a rounded tip, $52.5\text{--}64 \times 11.5\text{--}17\text{ }\mu\text{m}$.

COMMENTS—Among the known species, *Blodgettia saprophytica* slightly resembles *B. aquatica* and *B. indica* in conidial shape. However, the conidia of *B. aquatica* ($32\text{--}52 \times 15\text{--}25\text{ }\mu\text{m}$, 3–5-septate; Udaiyan 1992) and *B. indica* ($20\text{--}50 \times 7.5\text{--}17.5\text{ }\mu\text{m}$, 3–5(–6)-septate; Subramanian 1954) are smaller and have fewer septa than those of *B. saprophytica*. In addition, the conidia of *B. aquatica* and *B. indica* are not covered by a mucilaginous tunica.

Uberispora tropicalis Bhat & W.B. Kendr., Mycotaxon 49: 73, 1993.

FIG. 2

COLONIES on natural substrate effuse, brown, velvety. CONIDIOPHORES mononematous, erect, straight or slightly flexuous, rarely branched, pale brown, 3–7-septate, $40\text{--}120 \times 2.5\text{--}4.8\text{ }\mu\text{m}$. CONIDIOGENOUS CELLS integrated, terminal, monoblastic, cylindrical, almost colorless, $8\text{--}18 \times 0.85\text{--}2.6\text{ }\mu\text{m}$. CONIDIA solitary, dry, septate, $17\text{--}22\text{ }\mu\text{m}$ diam, with a central thick-walled, dark brown, smooth, angular cell, $14\text{--}18\text{ }\mu\text{m}$ diam, and 3 lateral, almost colorless, conical cells with obtuse apices and truncate bases $7\text{--}14 \times 8.5\text{--}12\text{ }\mu\text{m}$, with a conico-truncate basal cell $1.5\text{--}4.5 \times 1.3\text{--}2.6\text{ }\mu\text{m}$, often carrying the upper

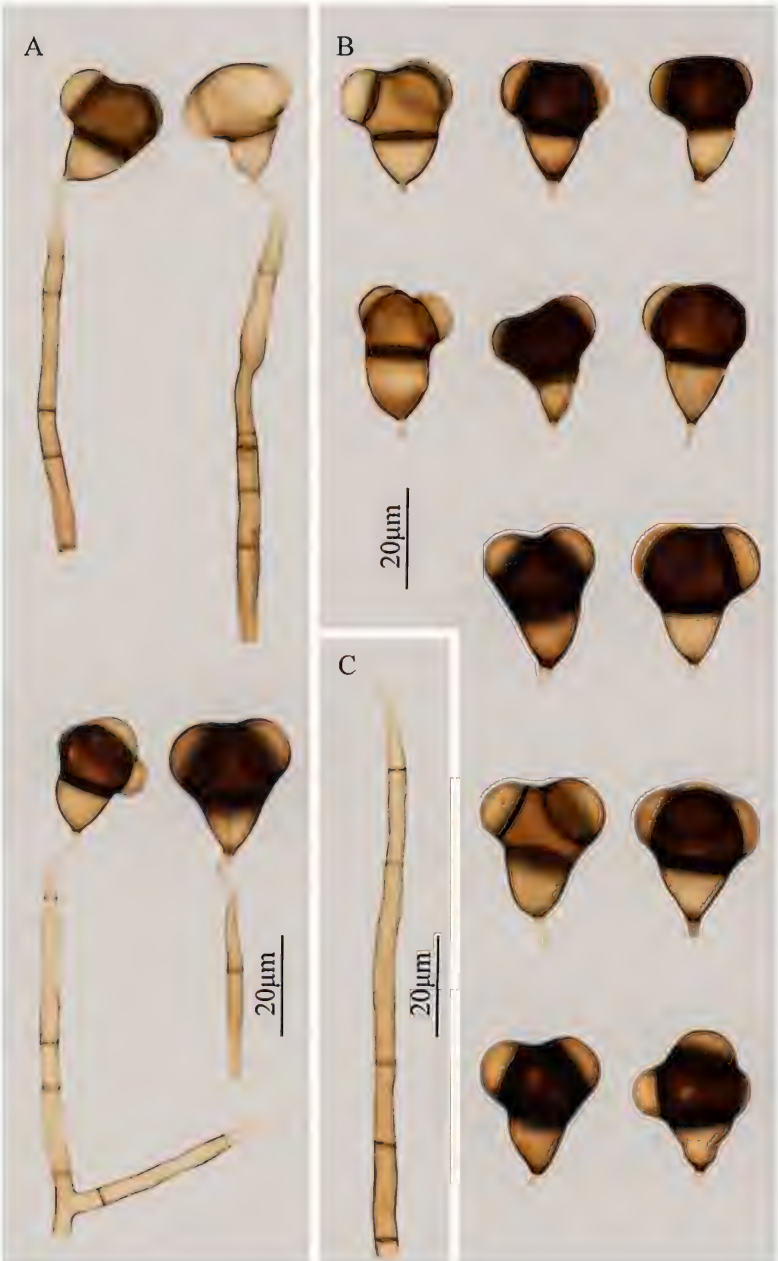


FIG. 2. *Uberispora tropicalis* (HSAUP H9783). A. Conidiophores, conidiogenous cells, and conidia. B. Conidia. C. Conidiophore and conidiogenous cell.

portion of the conidiogenous cell as a basal frill, seceding rhexolytically, with seceding conidia sometimes remaining attached to the side of the conidiophore by a wall remnant.

SPECIMEN EXAMINED: CHINA, GUANGXI PROVINCE: subtropical forest of Lengshui, on dead stems of unidentified broadleaf tree, 16 Nov. 2015, J.M. Gao (HSAUP H9783).

COMMENTS—*Uberispora tropicalis* is reported for the first time from China. The Chinese specimen closely matches the original description by Bhat & Kendrick (1993), except that the Chinese specimen has a curved and much narrower conidiogenous cell (0.85–2.6 µm vs 2.5–3.5 µm). However, in our opinion they are basically the same species.

Acknowledgments

The authors express gratitude to Dr. Rafael F. Castañeda-Ruiz and Dr. De-Wei Li for serving as pre-submission reviewers and for their valuable comments and suggestions. This project was supported by the National Natural Science Foundation of China (Nos. 31093440, 31230001, 31493010, 31493011, 31200013) and the Ministry of Science and Technology of the People's Republic of China (Nos. 2006FY120100).

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New record of *Beauveria pseudobassiana* from Morocco

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ABSTRACT—Two species of *Beauveria* were identified from isolates obtained from endemic *Argania spinosa* forests in Morocco, using both morphological characteristics and molecular data. Although isolates exhibited similar reproductive structures, ITS-rDNA based phylogenetic analysis grouped the Moroccan isolates into two clades with known sequences of either *B. bassiana* or *B. pseudobassiana*. Morphological comparison of the colonies distinguished the two groups of isolates, in full agreement with the ITS phylogenetic analysis. *Beauveria pseudobassiana* is recorded for the first time in Morocco.

KEY WORDS—*Ascomycetes*, entomopathogenic fungus, taxonomy

Introduction

Beauveria Vuill. (*Cordycipitaceae*, *Hypocreales*) is a genus of cosmopolitan entomopathogenic fungi found in various types of habitats and ecosystems (Meyling & Eilenberg 2007). Its species are known to infect a wide range of insects from many different orders in natural and agricultural environments (Roy et al. 2010). Preparations from *B. bassiana* have been developed and commercialized as biological control agents (Zimmermann 2007). Other *Beauveria* species hold great potential for the discovery of mycoinsecticidal effect (Thomas & Read 2007) and the production of different bioactive and pathogenetic compounds (Ames & Walsh 2010). Traditionally *Beauveria* species have been identified principally by conidial morphology. However,

the extensive overlap in morphological characters complicates and limits their usefulness for species identification. Furthermore, the discovery of cryptic species using molecular markers (Rehner & Buckley 2005, Ghikas et al. 2010, Rehner et al. 2011, Meyling et al. 2012) makes species identification a challenge within the genus (Fisher et al. 2011). Accordingly, accurate identification, including phylogenetic analyses, has been significantly improved using molecular methods (Rehner et al. 2011).

Through ITS and EF1- α sequence analyses, Rehner & Buckley (2005) demonstrated that the morphospecies *B. bassiana* was not monophyletic but comprised two morphologically indistinguishable clades that have been recently recognized as separate species (Rehner et al. 2011, Meyling et al. 2012). Recently, robust molecular phylogenies based on multi-loci sequences have resolved 12 well-supported terminal lineages within *Beauveria* (Rehner et al. 2011). Similarly, recent sequence analyses have resulted in the recent description of four new species (Zhang et al. 2012, Chen et al. 2013, Agrawal et al. 2014, Robène-Soustrade et al. 2015).

In a previous study of entomopathogenic fungi in forests of endemic *Argania spinosa* (L.) Skeels (*Sapotaceae*) in Morocco, *Beauveria* and *Metarhizium* species were found but only a few isolates were identified as *B. bassiana* using molecular analysis of the internal transcribed spacer regions of ribosomal DNA (ITS-rDNA) sequences (Imoulan et al. 2011). The present study aims to adapt the phylogenetic species recognition concept (Rehner et al. 2011) by identifying the strains obtained from the Moroccan forests based on both morphological and molecular analyses.

Materials & methods

Fungal isolates, growth conditions, and morphological observation

Beauveria isolates were recovered from soil samples collected from *Argania spinosa* forests in Morocco using a *Galleria mellonella* baiting method and maintained on Potato Dextrose Agar (PDA) slants at 4°C in complete darkness (Imoulan et al. 2011). The designation of isolates and their geographical origin are given in TABLE 1. To establish monosporic cultures, conidial suspensions of 1×10^5 conidia ml⁻¹ were prepared from fungal cultures grown on PDA for 2 weeks and plated on PDA plates. The single colony propagated from single conidia was transferred into a new PDA dish and incubated at 25°C. Dried agar culture vouchers were deposited in Herbarium Mycologium, Chinese Academy of Sciences, Beijing, China (HMAS).

Microscopic measurements of conidia were taken from slide-cultures produced by inoculating a small amount of mycelium on a block of the appropriate nutrient agar overlaid by a cover slip. Images were acquired using an AxioCam MRC digital camera on a Zeiss AxioScope microscope. Microscopic measurements were performed with

TABLE 1. *Beauveria* and *Cordyceps* strains and ITS sequences used in phylogenetic analysis. GenBank numbers in bold are newly generated sequences.

SPECIES	STRAIN	LOCALITY	HOST/SUBSTRATE	GENBANK NO.
<i>B. pseudobassiana</i>	2706	Morocco	Soil	KU364339
	2629	Morocco	Soil	KU364340
	2634	Morocco	Soil	KU364341
	2640	Morocco	Soil	KU364342
	2649	Morocco	Soil	KU364343
	2650	Morocco	Soil	KU364344
	2653	Morocco	Soil	KU364345
	2659	Morocco	Soil	KU364346
	2660	Morocco	Soil	KU364347
	2661	Morocco	Soil	KU364348
	2687	Morocco	Soil	KU364349
	2689	Morocco	Soil	KU364350
	2645	Morocco	Soil	KU364351
	ARSEF1855	Canada	<i>Coleoptera: Scolytidae</i>	HQ880796
	ARSEF3405 (T)	USA	<i>Lepidoptera: Tortricidae</i>	AY532022
	ARSEF6229	China	<i>Coleoptera: Scolytidae</i>	HQ880799
<i>B. bassiana</i>	2721	Morocco	Soil	KU364352
	2718	Morocco	Soil	KU364353
	ARSEF1040	Japan	<i>Lepidoptera: Bombycidae</i>	AY531972
	ARSEF751	Vietnam	<i>Coleoptera: Chrysomelidae</i>	AY532045
	ARSEF1564 (T)	Italy	<i>Lepidoptera: Arctiidae</i>	HQ880761
<i>B. australis</i>	ARSEF4622	Australia	<i>Orthoptera: Acridiidae</i>	HQ880788
	ARSEF4598 (T)	Australia	Soil	HQ880789
<i>B. brongniartii</i>	ARSEF617 (T)	France	<i>Coleoptera: Scarabaeidae</i>	HQ880776
	ARSEF7058	USA	<i>Hymenoptera: Formicidae</i>	HQ880773
	ARSEF7517	Japan	<i>Coleoptera: Scarabaeidae</i>	HQ880767
<i>B. asiatica</i>	ARSEF4850 (T)	Korea	<i>Coleoptera: Cerambycidae</i>	AY531936
	ARSEF4384	China	<i>Coleoptera: Scarabaeidae</i>	AY532026
<i>B. sungii</i>	ARSEF1685 (T)	Korea	<i>Coleoptera: Scarabaeidae</i>	AY531990
	ARSEF7279	Korea	<i>Coleoptera: Scarabaeidae</i>	HQ880813
<i>B. malawiensis</i>	ARSEF7760	Malawi	<i>Coleoptera: Cerambycidae</i>	DQ376247
	BCC17613	Australia	—	HQ880824
<i>B. vermiconia</i>	ARSEF2922 (T)	Chile	Soil	AY532012
<i>B. caledonica</i>	ARSEF2251	Brazil	<i>Coleoptera</i>	AY532003
	AESEF2567 (T)	Scotland	Soil	AY532006
<i>B. amorpha</i>	ARSEF2641 (T)	Brazil	<i>Hymenoptera: Formicidae</i>	AY532008
	ARSEF4149	Australia	<i>Coleoptera: Scarabaeidae</i>	HQ880804
<i>B. rudraprayagi</i>	MTCC8017 (T)	India	Silkworm	JQ266173
<i>B. varroae</i>	ARSEF8257 (T)	France	<i>Acari: Varroidae</i>	HQ880800
	ARSEF8259	France	<i>Acari: Varroidae</i>	HQ880801
<i>B. sinensis</i>	RCEF3903 (T)	China	<i>Lepidoptera: Geometridae</i>	HQ270152
<i>B. lii</i>	RCEF5500 (T)	China	<i>Coleoptera: Coccinellidae</i>	JN689372
<i>B. kipukae</i>	ARSEF7032 (T)	USA	<i>Homoptera: Delphacidae</i>	HQ880803
<i>C. militaris</i>	ARSEF5050	USA	<i>Lepidoptera</i>	HQ880829

AxioVision Rel. 4.6 software (Zeiss, Welwyn Garden City, UK). Length : width ratios are given as Q, and mean values are indicated by L^m (length), W^m (width), and Q^m (Q).

Genomic DNA extraction, PCR and sequencing

Isolates recovered from single conidia were grown as mycelia for 2 weeks in 250-ml flasks containing 100ml of potato dextrose broth. Cultures were then shaken at 150 rpm on a rotary shaker at 25°C for 7d in darkness. The mycelial samples were pelleted from liquid cultures by centrifugation for 10 min at 3500 rpm (Eppendorf AG Centrifuge, Hamburg), subsequently washed twice with sterile double distilled water, and then lyophilized and stored at -80°C.

For each isolate, 400–500mg of lyophilized mycelium was ground in a sterile mortar using a pestle. Total genomic DNA was extracted following the modified CTAB method described by Yao et al. (1999). The mixtures were incubated for 2 hours at 65°C, then extracted twice with 24 : 1 chloroform : isoamyl alcohol, and centrifuged at 10,000 rpm at 4°C for 15 min. The supernatant was transferred to a clean tube and mixed with 1/10 volume of sodium acetate (3 M, pH 5.2). Total genomic DNA was precipitated by adding 2/3 (v/v) of cold isopropanol and chilled overnight at -20°C. DNA was recovered by centrifugation at 10,000 rpm, 4°C for 10 min and washed twice with 70% alcohol, dried at room temperature and resuspended in TE buffer (10Mm Tris-HCl, pH 8.0; 1mM EDTA, pH 8.0). The extracted DNA was stored at -20°C until use.

Genomic DNA was used as template for PCR amplification of ITS region using universal primers ITS5/ITS4 (White et al. 1990). The PCR reactions were performed in a final volume of 50 µl containing 25µl 2× Taq PCR Master Mix (Tiangen Biotech Co., Ltd, China), 0.5 µl of each primer (10 µM), 1 µl of genomic DNA and 23 µl of RNase-Free water. The PCR reactions were performed in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems) as follows: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 45 s and final extension step of 72°C for 8 min. PCR products were checked by electrophoresis on an agarose gel (1%) at 100 V in 0.5× TAE buffer (40 mM Tris-Acetic acid, pH 8.0, 1 mM EDTA), and sequenced with the same primer pairs. The sequencing was performed at Beijing Genomics Institute (Beijing, China) by using an Applied Biosystems 3730 Analyzer capillary sequencer (Foster City, CA, USA). All sequences generated in this study (KU364339–KU364353) were submitted to GenBank (TABLE 1).

Phylogenetic and data analyses

Only two (of 100) *B. bassiana* strains sequenced for ITS from Morocco were used as representatives, while all Moroccan strains identified as *B. pseudobassiana* were included in the analyses. Also included were 29 representative *Beauveria* sequences and one sequence of *Cordyceps militaris* (ARSEF 5050; as outgroup) retrieved from GenBank (TABLE 1). The sequences were aligned using ClustalW Multiple alignment tool (Thompson et al. 1997) and edited manually to avoid some obvious misalignment using BioEdit ver. 7.2.5 (Hall 1999).

The sequence data set was analyzed for maximum parsimony (MP) and Bayesian inference (BI). MP analyses were conducted with PAUP* 4.0b10 (Swofford 2003) and

executed with 1000 replicates of heuristic search option of random sequence additions with branch swapping algorithm by tree bisection reconnection (TBR). Alignment gaps were treated as missing data, and all characters were unordered and equally weighted. Branch support was estimated by bootstrap analysis with 1,000 replicates (Felsenstein 1985) executing the same search strategy as described above. Bayesian inference (BI) analyses were carried out in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) using a general time-reversible model and gamma-distributed rate variation to account for rates of variation across sites, with a proportion of invariant sites. The remaining parameters were default values. One tree was saved every 1,000 generations from a total of 5,000,000 Markov chain Monte Carlo (MCMC) generations; the first 25% of the trees were discarded as burn-in. Clades with bootstrap values for MP $\geq 50\%$ and Bayesian posterior probability $\geq 70\%$ are labeled above the nodes in the phylogenetic tree.

Results

Morphological observation

Microscopically *Beauveria* isolates from Morocco appeared typical to those described elsewhere (e.g., Humber 1997, Rehner et al. 2005). The colony characteristics were: white or pale-yellow mycelium closely (or not) appressed to agar surface, becoming farinaceous during sporulation and the reverse side uncoloured or yellowish white (Figs 1, 2). Conidiophores appeared like dense spherical clusters of subglobose to flask-shaped conidiogenous cells extending upward with an elongating sympodial denticulate rachis (zigzag appearance) giving rise to sessile, hyaline, holoblastic smooth conidia (Figs 3, 4). Two isolate groups were morphologically distinguished based on the colony colour as shown by aerial hyphae during conidiation: off-white and loosely floccose vs. yellowish-brown and densely farinaceous (Figs 1, 2).

Phylogenetic analyses

The amplification of ITS-rDNA region produced a uniform fragment size of 560bp. Using Blast search, the sequences exhibited high sequence homology to those named *B. bassiana* or *B. pseudobassiana* in GenBank.

The ITS dataset comprised 571 characters after the exclusion of ambiguous positions from both ends. There were 488 constant, 37 parsimony-uninformative, and 46 parsimony-informative characters. The heuristic search yielded six most-parsimonious trees with tree length (L) = 139, consistency index (CI) = 0.719, homoplasy index (HI) = 0.281, retention index (RI) = 0.807. MP and BI phylogenetic analyses produced trees with similar topologies that resolved most *Beauveria* lineages in separate terminal branches (Fig. 9). Independently of the algorithm used for phylogenetic analyses, *B. brongniartii* and *B. australis* were not well distinguished as unique clusters based on ITS-rDNA sequences.

Moroccan strains of *Beauveria* were grouped into two individual terminal clades corresponding to *B. bassiana* and *B. pseudobassiana* with MP bootstrap support $\geq 76\%$ and BI posterior probabilities $\geq 99\%$ (FIG. 9). These two groups corresponded well to their colony colours, off-white in the *B. bassiana* clade and yellowish-brown in the *B. pseudobassiana* clade.

Taxonomy

Imoulan et al. (2011) previously reported *Beauveria bassiana* from Morocco. Here we describe *B. pseudobassiana* as a new record for Morocco.

Beauveria pseudobassiana S.A. Rehner & Humber, Mycologia 103: 1068

(2011)

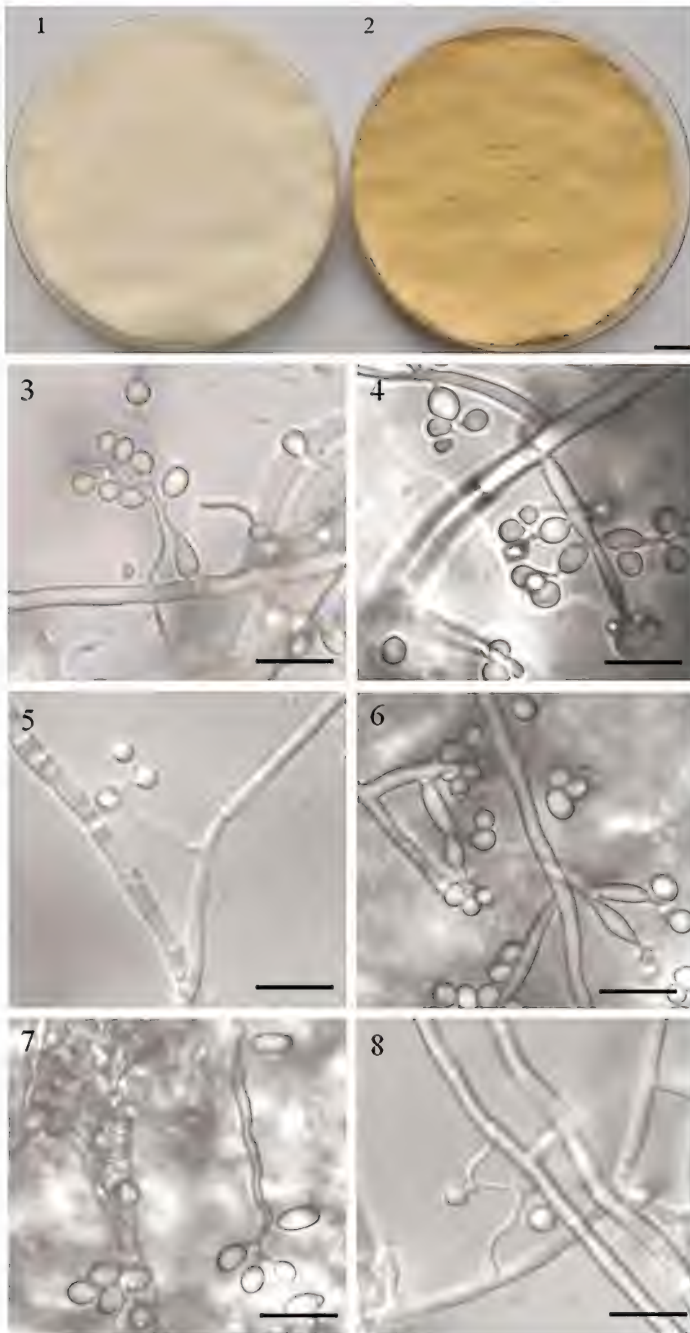
FIGS 2–8

Colonies growing fairly well on PDA at 25°C in the dark, reaching a diameter of 28–37 mm after 7 days incubation, and attaining a diameter of 36–42 mm in 10 days; velutinous to cottony with an overgrowth of aerial hyphae up to the agar surface, white first and changing to yellowish-brown or pale yellow; becoming less powdery in older cultures and rarely with a dense farinaceous surface layer. Reverse side yellow with white margin. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1–3 μm wide. Conidiogenous cells solitary but usually consisting of dense lateral clusters, base subspherical to flask-shaped (sometimes ampulliform), $2.0\text{--}9.0 \times 1.5\text{--}3 \mu\text{m}$, apex with an indeterminate denticulate rachis, produced laterally on aerial hyphae or from subtending cells. Conidia globose to subglobose, $2\text{--}4 \times 1\text{--}3.5 \mu\text{m}$, $Q = 1\text{--}2.5$ ($L^m = 2.6 \mu\text{m}$, $W^m = 2.1 \mu\text{m}$, $Q^m = 1.4$), rarely ellipsoid; hyaline, walls thin and smooth; produced from conidiogenous cells and occasionally directly from hyphal tips or laterally from hyphae.

MATERIAL EXAMINED: MOROCCO, OUJDA; from soil under *Argania spinosa* tree, 23 March 2009, coll. A. Imoulan, strain numbers 2629, 2634, 2640, 2645, 2649, 2650, 2653, 2659, 2660, 2661 (HMAS 254118–254127, dried agar cultures); Tamanar, 28 March 2009, coll. A. Imoulan, strain numbers 2687, 2689 (HMAS 254128–254129, dried agar cultures); Amskroud, 29 March 2009, coll. A. Imoulan, strain number 2706 (HMAS 254131, dried agar culture).

REMARKS: *Beauveria pseudobassiana* from Morocco differed from *B. bassiana* by its colony surface, which is yellowish-brown to pale yellow (FIG. 2) becoming less powdery when old, and by its occasional production of ellipsoid conidia (FIG. 7). The colony surface of *B. bassiana* is usually white to off-white

FIGS 1–8. *Beauveria bassiana* (strain 2718): 1. colony and aerial hyphae. *Beauveria pseudobassiana* (strain 2629): 2. colony and aerial hyphae; 3–8. conidiophores, conidiogenous cells, and conidia — note conidiogenous cell produced from subtending cells (6), conidia forming from a hyphal tip (7), and conidia produced directly from mycelium (8). Scale bars: 1, 2 = 10 mm; 3–8 = 5 μm .



(FIG. 1) becoming powdery when old, owing to the production of a large number of globose to subglobose conidia.

Discussion

The study of mycology in Morocco is less developed with few works being published on Moroccan fungi, particularly on entomopathogenic fungi (Imoulan et al. 2011, Imoulan & El Meziane 2013). The new species record for Morocco reported here demonstrates that *B. pseudobassiana* occurs in *A. spinosa* forests along with other entomopathogenic fungi such as *B. bassiana*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus* identified previously by Imoulan et al. (2011).

As shown in the most recent taxonomic revision based on the phenotypic criteria combined with molecular evidence (Rehner & Buckley 2005, Rehner et al. 2011), *B. pseudobassiana* and *B. bassiana* comprise a morphospecies group, morphologically indistinguishable yet phylogenetically only distantly related. Interestingly, our morphological observation nonetheless revealed that characters of both colony and conidial spores were sufficient to discriminate the two species. As these morphological distinctions have not been mentioned elsewhere, it is unclear whether our experience represents an unusual case or if it is possible to find additional characters to distinguish *Beauveria* species morphologically. It would be very useful if such characters could support DNA-based phylogenies in *Beauveria*.

In the current study, ITS-rDNA-based phylogenetic analyses succeed in distinguishing *B. bassiana* and *B. pseudobassiana* among the species analyzed. Because *Beauveria* includes cryptic species that cannot be resolved solely by ITS analyses, multilocus-based phylogenies are becoming increasingly important for accurate assignment of strains to specific clades. Molecular phylogenies involving multiple genes of translation elongation factor-1 α (TEF), RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2), and the Bloc nuclear intergenic have become well-established for determining unique terminal lineages for all *Beauveria* species (Rehner et al. 2011, Zhang et al. 2012, Chen et al. 2013, Agrawal et al. 2014, Robène-Soustrade et al. 2015). However, our ITS sequence analyses performed well in distinguishing 12 of the 15 species included, with *B. australis* and *B. brongniartii* grouping in a single unresolved clade, and *B. rudraprayagi* (MTCC8017) grouping with *B. pseudobassiana* (FIG. 9). Although the additional genes are required in resolving closed related species, ITS sequence is still powerful in identifying most of the known species of *Beauveria* as shown in FIG. 9.

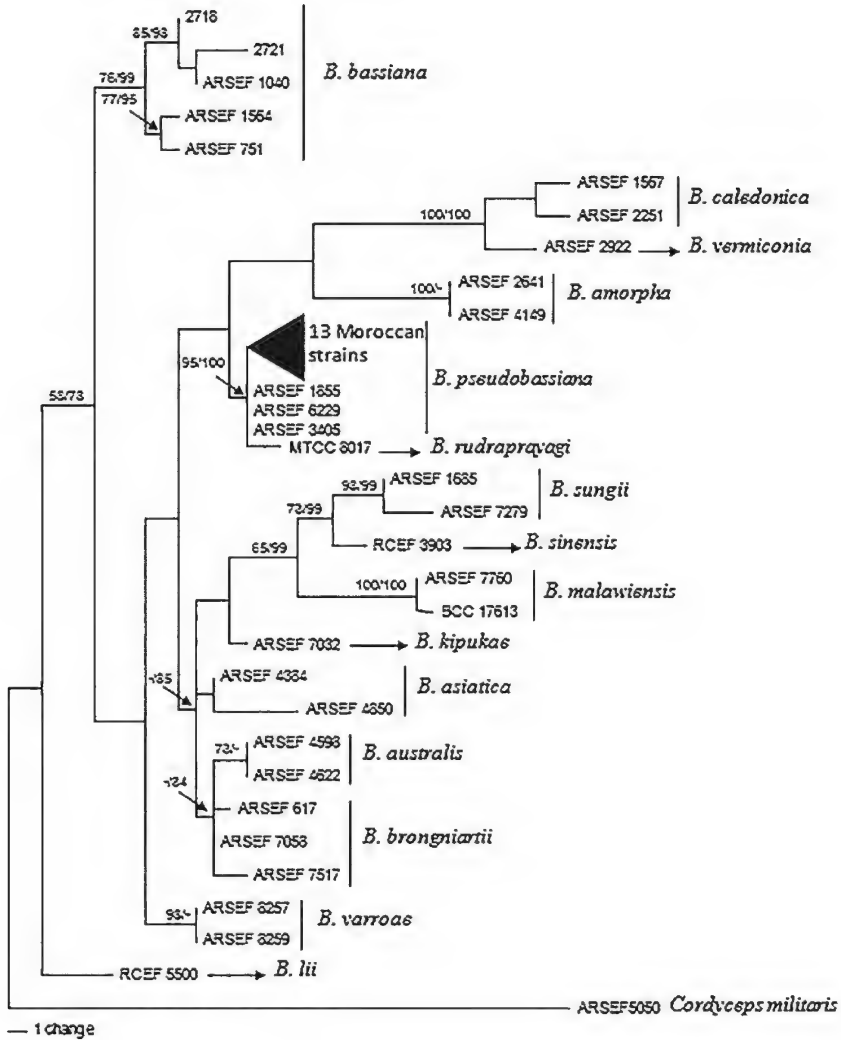


FIG. 9. Phylogenetic tree of *Beauveria* based on Maximum parsimony and Bayesian inference of the ITS-rDNA sequences. Bootstrap values $\geq 50\%$ and posterior probabilities $\geq 70\%$ are labeled above branches and separated by /. Terminal clades are labeled according to ARSEF accession numbers of individual isolates reported in Rehner et al. (2011) except RCEF5500 and RCEF3903 (Zhang et al. 2012, Chen et al. 2013).

Acknowledgments

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New records of *Graphis* from Cameroon, with a key to African species of *Graphis*

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ABSTRACT—New records of *Graphis* species are reported from Cameroon, West Africa: *G. ajarekarii*, *G. alboglaucescens*, *G. brahmanensis*, *G. daintreensis*, *G. exalbata*, *G. gloriosensis*, *G. gonimica*, *G. handelii*, *G. immersella*, *G. novopalmicola*, *G. pseudoaquilonia*, and *G. supracola*. The material was collected in the tropical rain forests of Mount Cameroon. The diagnostic characters of the species are briefly discussed and illustrated. An artificial key is provided to facilitate identification of *Graphis* species known from the African Palaeotropics.

KEY WORDS—corticolous, crustose lichens, taxonomy, *Graphidaceae*, *Ostropales*

Introduction

Mount Cameroon (ca. 4°N 9°E) is the highest mountain (4095 m) in West Africa, being located near the Gulf of Guinea in the Southwest Region of Cameroon (Fonge et al. 2005, Proctor et al. 2007). The Mount Cameroon region has a diversity of complex ecosystems due to frequent disturbances, (especially volcanic activities) that have made it possible to study different successional stages in a relatively confined area (Focho et al. 2010). The western rain forests of Africa, despite having luxuriant forests with high organismic diversity, are little explored in terms of lichen diversity (including the *Graphidaceae*) compared to the Eastern Palaeotropics in Asia.

In the course of an ongoing research project, crustose lichens, particularly graphidoid representatives, were collected from rainforests at different

elevations at Mount Cameroon. The thelotremoid lichens in the *Graphidaceae* have been exhaustively studied in the region (Frisch et al. 2006); however, no detailed account on graphidoid taxa from this region is available and only scattered reports from the African Palaeotropics exist in the literature for this group (Müller 1890, Staiger 2002, Lücking et al. 2009).

Here we report twelve new species representing the genus *Graphis* Adans. collected in the Mount Cameroon area: *G. ajarekarii*, *G. alboglauescens*, *G. brahmanensis*, *G. daintreensis*, *G. exalbata*, *G. gloriosensis*, *G. gonimica*, *G. handelii*, *G. immersella*, *G. novopalmicola*, *G. pseudoaquilonia*, and *G. supracola*. We also present an artificial key to the species of *Graphis* known from the African Palaeotropics.

Materials & methods

During January 2015 a field excursion organized by Prof. Andrew Enow Egbe, University of Buea, targeted one area in the Mount Cameroon region, Federal Republic of Cameroon. Lichen specimens are deposited in the lichen herbarium of the Korean Lichen Research Institute, Suncheon, South Korea (KoLRI), and duplicates are preserved in the Faculty of Science in the University of Buea, Cameroon. Samples were studied in CSIR-National Botanical Research Institute, Lucknow, India. The material was examined for morpho-anatomical and chemical features using a MSZ-TR dissecting microscope and a Leica DM 500 compound microscope. The protocol for color spot reaction tests and thin-layer chromatography (TLC) was derived from Orange et al. (2010). Thin-layer chromatography was performed in solvent systems A [toluene (180): 1, 4-dioxane (45): acetic acid (5)] and C [toluene (170): acetic acid (30)]. Lugol's solution (I) was used to check amyloidity of the ascospores. Illustrations were prepared using Corel Draw (vers. 12).

Taxonomy: new records

Graphis ajarekarii Patw. & C.R. Kulk., Norw. J. Bot. 26: 45, 1979.

PL. 1A

Thallus epiperidermal, slightly uneven, dull to $\pm$ glossy, ≤ 250 μm thick; prothallus dark brown; lirellae immersed to erumpent, strongly branched, 1–2 cm long, with a lateral to apically thin complete thalline margin; disc concealed; labia entire, epruinose; proper exciple laterally carbonized becoming completely carbonized in old lirellae, 50–70 μm thick; epihymenium brownish and granular, 15–20 μm high; hymenium hyaline, clear, 90–100 μm high; subhymenium 20–30 μm high; asci 8-spored, 100–130 $\times$ 20–25 μm ; ascospores fusiform, transversely septate, 8–10-locular, amyloid, 28–48 $\times$ 8–11 μm .

CHEMISTRY—Norstictic and stictic acids detected with TLC.

DISTRIBUTION & ECOLOGY—Found growing in association with other graphidoid taxa on rough barked trees at higher altitudes.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°09'01"N 9°17'02"E, on bark, 25 February 2015, J–S. Hur CR150020 (KoLRI).

REMARKS—*Graphis filiformis* Adaw. & Makhija, which otherwise resembles *G. ajarekarii* in producing norstictic acid and small ascospores, lacks stictic acid. *Graphis ajarekarii* was previously known from the Eastern Palaetropics (Lücking et al. 2009).

Graphis alboglaucescens Adaw. & Makhija, Mycotaxon 99: 312, 2007. PL. 1B

Thallus epiperidermal, ecorticate, farinose, greenish-white to glaucous green; lirellae immersed, elongate (1–2 cm long) and irregularly branched with lateral thalline margin; disc slightly exposed, white pruinose (*scripta*-morph); labia entire; proper exciple apically carbonized, 30–40 µm thick; epihymenium brownish, granular, 10–15 µm high; hymenium hyaline and clear, 90–100 µm high; subhymenium indistinct; asci 8-spored; ascospores fusiform, transversely septate, 5–10-locular, amyloid, 24–26 × 4–6 µm.

CHEMISTRY—Norstictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Found in small patches on hard and rough barked trees in evergreen forests at higher altitudes.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°07'26"N 9°11'17"E, alt. ca. 1200 m on bark, 26 February 2015, J–S. Hur CR150067 (KoLRI).

REMARKS—In its anatomy, *G. alboglaucescens* resembles *G. glaucescens* Fée, which is distinguished by its lack of secondary metabolites and occasionally striate labia. *Graphis alboglaucescens* was originally described as producing lirellae covered up to the top by a thalline margin (Adawadkar & Makhija 2007), a character Lücking et al. (2009) later defined as a lateral thalline margin. The species exhibits a pantropical distribution (Lücking et al. 2009).

Graphis brahmanensis Aptroot, Lichenologist 41: 434, 2009. PL. 1C

Thallus epiperidermal, thin, pale-brown, fawn to off-white, 80–100 µm, evanescent, indistinctly corticate reflecting bark; algal layer continuous, 28–30 µm thick; medulla mostly endoperidermal; lirellae prominent, 1–1.5 cm long, sparsely and irregularly branched with basal thalline margin (*striatula*-morph); labia striate (3–4-striations), epruinose; proper exciple laterally carbonized, 50–100 µm thick; epihymenium brownish, 10–15 µm high; hyaline and clear; hymenium 90–100 µm high; subhymenium 10–15 µm high; asci 8-spored; ascospores fusiform, transversely septate, 5–8-locular, amyloid, 25–30 × 6–8 µm.

CHEMISTRY—Stictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Found growing in association with other corticolous lichen taxa at low altitudes on smooth barked trees of tropical rain forests.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°05'41"N 9°22'24"E, alt. ca. 100 m, on bark, 26 February 2015, J–S. Hur CR150072 (KoLRI).

REMARKS—In most characters, *G. brahmanensis* resembles *G. vittata* Müll. Arg., which differs in producing lirellae with a lateral thalline margin. The otherwise similar *G. duplicata* Ach. lacks secondary compounds.

Graphis brahmanensis was previously known from the Neotropics, (particularly in the southeastern USA) and Eastern Palaeotropics (Lücking et al. 2009).

Graphis daintreensis (A.W. Archer) A.W. Archer, Telopea 11: 72, 2005. PL. 1D

Thallus epiperidermal, silver-gray to greenish gray or whitish-gray, off-white, glossy, uneven, 200–250 µm thick, corticate; cortex continuous, 20–30 µm thick; algal layer 40–140 µm thick, embedded with large and small crystals; indistinct to endoperidermal medulla; indistinct to off white prothallus; lirellae elongate (1–2 cm long), densely and irregularly branched, prominent with basal thalline margin (*hossei*-morph); disc concealed; labia entire and epruinose; proper exciple laterally carbonized, 40–60 µm thick; epihymenium brownish, crystalline, 15–20 µm high; hymenium hyaline and clear, 130–150 µm high; subhymenium indistinct; asci broadly clavate, 1-spored, 110–120 × 25–30 µm; ascospores muriform, multi-celled, amyloid, 60–90 × 20–25 µm.

CHEMISTRY—No chemicals detected with TLC.

DISTRIBUTION & ECOLOGY—In Cameroon, this taxon was collected from the rainforests at very low altitudes, and was found growing in large patches on rough barked trees.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°04'09"N 9°22'36"E, alt. ca. 30 m, on bark, 26 February 2015, J–S. Hur CR150081 (KoLRI).

REMARKS—*Graphis nanodes* Vain., which resembles *G. daintreensis* in most taxonomic characters, differs in its lirellae with lateral thalline margins (*lineola*-morph) and smaller ascospores (≤50 µm long).

Graphis daintreensis was previously known only from the Eastern Palaeotropics (Lücking et al. 2009).

Graphis exalbata Nyl., Lich. Ins. Guin.: 28, 1889. PL. 1E

Thallus epiperidermal, whitish, smooth to slightly uneven, glossy, indistinctly verruculose, continuous, ecorticate, 100–200 µm, and embedded with crystals;

algal layer ≤ 90 μm thick, embedded with crystals; medulla endoperidermal; prothallus indistinctly whitish; lirellae $\pm$ immersed to erumpent, elongate (1–2 cm long), radially branched with lateral thalline margin; disc concealed; labia indistinctly striate, white pruinose (*glaucescens*-morph); proper exciple apically carbonized, 30–60 μm thick; epihymenium grayish crystalline, 5–10 μm high; hymenium hyaline and clear, 90–100 μm high; subhymenium 15–20 μm high; asci 8-spored, 90–100 $\times$ 10–15 μm ; ascospores transversely septate, 8–10-locular, amyloid, 30–38 $\times$ 8–9 μm .

CHEMISTRY—Norstictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Found growing in large patches on thin and smooth barked trees in evergreen forests.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°09'01"N 9°17'02"E, on bark, 25 February 2015, J–S. Hur CR150024 (KoLRI).

REMARKS—*Graphis eburnea* Adaw. & Makhija, which is morphologically and chemically close to *G. exalbata*, is distinguished by its epruinose labia (*nigrosina*-morph).

Lücking et al. (2009) previously reported *G. exalbata* from the Palaeotropics.

Graphis gloriosensis A.W. Archer & Elix, Telopea 11: 454, 2007

Pl. 1F

Thallus epiperidermal, thin, pale-brown, fawn to off-white, indistinctly corticate 50–100 μm thick, reflecting bark; algal layer continuous, 28–30 μm thick; medulla mostly endoperidermal; lirellae prominent, sparsely and irregularly branched, 1–1.5 cm long with lateral thalline margin (*marginata*-morph); labia entire, epruinose; proper exciple completely carbonized, 50–100 μm thick; epihymenium brown, granular, 10–15 μm high; hymenium hyaline and densely interspersed (with oil droplets), 90–100 μm high; subhymenium 10–15 μm high; asci 8-spored; ascospores fusiform, transversely septate, 10–15-locular, amyloid, 40–70 $\times$ 10–15 μm .

CHEMISTRY—Stictic and hypostictic acids detected with TLC.

DISTRIBUTION & ECOLOGY—Collected from tree bark in evergreen forests at higher altitudes.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°07'40"N 9°11'54"E, alt. 1150 m, on bark, 26 February 2015, J–S. Hur CR150060 (KoLRI).

REMARKS—In thallus color and texture and in chemistry *G. gloriosensis* superficially resembles *G. brahmanensis*, which differs in its striate labia, laterally carbonized proper exciple, and clear hymenium.

Lücking et al. (2009) reported *G. gloriosensis* from the Eastern Paleotropics.

Graphis gonimica Zahlbr., Ann. Mycol. 30: 431, 1932.

PL. 1G

Thallus epiperidermal, whitish-gray, silver-gray, white or dark-gray, glossy, ≤ 200 μm thick; cortex 30–40 μm thick, continuous; algal layer 45–60 μm thick; medulla white, crystalline, 60–100 μm thick; prothallus dark brown; lirellae immersed, short (0.5–1 cm long) and scarcely furcated with lateral thalline margin (*lineola*- or *deserpens*-morph); disc concealed; labia entire and epruinose; proper exciple $\pm$ completely carbonized, 60–150 μm thick; epihymenium brownish, granular, 10–15 μm high; hymenium densely inspersed (with oil droplets), 140–160 μm high; subhymenium indistinct; asci 8-spored; ascospores fusiform, transversely septate, 6–12-locular, amyloid, $30\text{--}50 \times 7\text{--}9$ μm .

CHEMISTRY—Norstictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Found growing in small patches associated with *Glyphis cicatricosa* Ach. and *Arthonia* sp. on uneven and thick barked trees in evergreen forests.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, $4^{\circ}09'01''\text{N}$ $9^{\circ}17'02''\text{E}$, on bark, 25 February 2015, J–S. Hur CR150035, CR150036 (KoLRI).

REMARKS—The morphologically similar *G. cincta* (Pers.) Aptroot differs in its laterally carbonized proper exciple.

Lücking et al. (2009) previously reported *G. gonimica* from the Paleotropics.

Part of our material sometimes produces stictic acid and atranorin (in traces) as additional compounds, but we refrain from proposing a taxon new to science until further collection and evaluation.

Graphis handelii Zahlbr., Symb. Sinic. 3: 44, 1930.

PL. 1H

Thallus epiperidermal, off-white, grayish-green to gray, 100–150 μm thick; cortex continuous, 20–30 μm thick; algal layer continuous, 30–40 μm thick; medulla white crystalline, epi- to endoperidermal, 50–80 μm thick; lirellae erumpent to prominent, short (≤ 5 mm long), simple to furcate; disc exposed and epruinose, brown; labia entire and epruinose; proper exciple laterally carbonized 20–50 μm thick; epihymenium brownish, 10–15 μm high; hymenium hyaline and inspersed (with oil droplets), 90–100 μm high; subhymenium 20–30 μm high; asci 8-spored, $70\text{--}90 \times 10\text{--}15$ μm ; ascospores fusiform, transversely septate, 5–9-locular, amyloid, $28\text{--}32 \times 5\text{--}7$ μm .

CHEMISTRY—Norstictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Collected from smooth barked trees in evergreen forests.

SPECIMENS EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°09'01"N 9°17'02"E, on bark, 25 February 2015, J–S. Hur CR150028, CR150038 (KoLRI).

REMARKS—*Graphis crebra* Vain. is similar but is distinguished by its white-pruinose disc.

Lücking et al. (2009) report a pantropical distribution for *G. handelii*.

Graphis immersella Müll. Arg., Bull. Herb. Boissier 3: 319, 1895. PL. 11

Thallus epiperidermal, off white, greenish-gray, ≤ 150 μm thick; cortex continuous, 30–50 μm thick; algal layer 30–40 μm thick; medulla endoperidermal; prothallus blackish; lirellae erumpent, short (≤ 1 cm long) and sparsely branched (*lineola*-morph) with lateral thalline margin; disc concealed; labia entire and epruinose; exciple laterally carbonized, 20–60 μm thick proper; epihymenium dark brown, crystalline, 10–15 μm high; hymenium hyaline and clear, 100–150 μm high; subhymenium indistinct; asci broadly clavate, 8-spored, 90–100 $\times$ 10–15 μm ; ascospores transversely septate, 8–11-locular, amyloid, 35–55 $\times$ 8–10 μm .

CHEMISTRY—Stictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Collected from rather smooth barked trees in evergreen forests.

SPECIMEN EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°07'41"N 9°12'20"E, on bark, 26 February 2015, J–S. Hur CR150057 (KoLRI).

REMARKS—*Graphis pinicola* Zahlbr. is morphologically similar to *G. immersella* but lacks lichen compounds.

Lücking et al. (2009) report a palaeotropical distribution for *G. immersella*.

Graphis novopalmicola A.W. Archer & Lücking, Lichenologist 41: 439, 2009. PL. 11

Thallus epiperidermal, pale-gray to yellowish-gray, dull, loosely corticate, 150–200 μm thick, continuous in large patches; cortex 20–30 μm thick embedded with crystals; algal layer continuous, 40–50 μm thick; medulla mostly endoperidermal and crystalline, 100–150 μm thick; prothallus whitish; lirellae erumpent to prominent, simple to furcate, short to elongate (1–1.5 cm long) with a thick lateral thalline margin (*subserpentina*-morph); concealed disc; entire and epruinose labia; proper exciple completely carbonized, ≤ 50 μm thick; epihymenium brownish crystalline, 10–15 μm high; hymenium densely inspersed (with oil droplets) 150–170 μm high; subhymenium indistinct to 20 μm high; asci broadly clavate, 1-spored; ascospores large, muriform, multicelled, amyloid, 90–110 $\times$ 20–30 μm .

CHEMISTRY—Norstictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Collected from smooth barked trees in evergreen forests near foothills.

SPECIMENS EXAMINED: CAMEROON. **SOUTHWEST PROVINCE:** Mount Cameroon, 4°04'09"N 9°22'36"E, alt. ca. 30 m, on bark, 26 February 2015, J-S. Hur CR150079, CR150080 (KoLRI).

REMARKS—*Graphis leprographa* Nyl., which resembles *G. novopalmicola* in having 1–2-spored asci and similar chemistry, differs in its very short and unbranched lirellae (*dussii*-morph).

Lücking et al. (2009) report a pantropical distribution for *G. novopalmicola*.

Graphis pseudoaquilonia Lücking, Lichenologist 44: 393, 2012. PL. 1K

Thallus epiperidermal, cartilaginous, grayish-green, dark-gray to gray, continuous, glossy, corticate, spreading in large wide patches; lirellae prominent, elongate (1–2 cm long), sinuous, irregularly branched with complete thalline margin; disc concealed; labia striate (*archeri*-morph); proper exciple completely carbonized, 90–100 µm thick; epihymenium brownish, granular, 10–15 µm high; hymenium hyaline and clear, 100–150 µm high; subhymenium 20–30 µm high; asci 2–6-spored; ascospores fusiform, terminally muriform, amyloid [(10–15-septate in the middle × 1–2 longitudinal septa per cell in the terminal segments)], 50–80 × 9–15 µm.

CHEMISTRY—Norstictic acid detected with TLC.

DISTRIBUTION & ECOLOGY—Found growing in large patches on uneven and thick barked trees in evergreen forests.

SPECIMENS EXAMINED: CAMEROON. **SOUTHWEST PROVINCE:** Mount Cameroon, 4°09'01"N 9°17'02"E, on bark, 25 February 2015, J-S. Hur CR150011, CR150025 (KoLRI).

REMARKS—*Graphis norvestitoides* Sutjaritt., which is otherwise similar to *G. pseudoaquilonia* in all characters, is distinguished by its strongly inspersed hymenium (Lücking et al. 2012).

Graphis pseudoaquilonia has previously been reported from the Neotropics and Eastern Palaeotropics (Lücking et al. 2009, Lücking et al. 2012).

Graphis supracola A.W. Archer, Aust. Syst. Bot. 14(2): 267, 2001. PL. 1L

Thallus whitish-gray to gray, greenish-gray, loosely corticate, ≤100 µm thick, smooth to slightly verruculose, spread in patches; cortex discontinuous ≤15 µm thick, overlying a discontinuous layer of crystals; algal layer 40–60 µm thick; medulla mostly endoperidermal; prothallus dark brown; lirellae immersed, simple to furcate, 1–2 cm long with lateral thalline margin; disc concealed to sometimes slightly open; labia entire and white pruinose (*caesiella*-morph);

proper exciple laterally carbonized, 30–50 µm thick; epihymenium brownish, 5–10 µm high; hymenium hyaline and clear, 50–100 µm high; subhymenium 35–45 µm high; asci 8-spored; ascospores fusiform, transversely septate, 6–10-locular, amyloid, $25\text{--}32 \times 7\text{--}10$ µm.

CHEMISTRY—Protocetraric acid detected with TLC.

DISTRIBUTION & ECOLOGY—Found growing on tree barks in evergreen forests at low altitudes.

SPECIMENS EXAMINED: CAMEROON. SOUTHWEST PROVINCE: Mount Cameroon, 4°09'01"N 9°17'02"E, on bark, 25 February 2015, J–S. Hur CR150023 (KoLRI); 4°05'41"N 9°22'24"E, alt. ca. 100 m, on bark, 26 February 2015, J–S. Hur 150069 (KoLRI).

REMARKS—This taxon can be confused with *Graphis distincta* Makhija & Adaw., which differs in additionally producing stictic and constictic acids.

Lücking et al. (2009) report a pantropical distribution for *G. supracola*.

Key to *Graphis* species recorded from the African Palaeotropics

Taxonomic key characters of *Graphis* species not included in our collections derived primarily from Lücking et al. (2009) and Broeck et al. (2014)

1. Lirellae orange-pigmented, prominent to sessile, short to elongate, sparsely to irregularly branched; ascospores terminally muriform, 70–120 × 10–15 µm; no lichen substances present *G. subchrysocarpa*
1. Lirellae not pigmented 2
2. Labia entire; hymenium clear or inspersed 3
2. Labia striate; hymenium clear 32
3. Proper exciple completely carbonized 4
3. Proper exciple apically to laterally carbonized 16
4. Hymenium inspersed 5
4. Hymenium clear 8
5. Ascospores muriform, 90–110 × 20–30 µm; norstictic acid present *G. novopalmiticola*
5. Ascospores transversely septate 6
6. Lichen substances absent; lirellae erumpent with complete thalline margin (*subserpentina*-morph); ascospores 18–20-locular, 100–120 × 14–18 µm *G. superans*
6. Lichen substances present 7
7. Stictic acid present; ascospores 10–15-locular, 50–90 × 10–15 µm *G. gloriosensis*
7. Norstictic acid present; ascospores 6–12-locular, 30–50 × 7–9 µm *G. gonimica*
8. Lichen substances present 9
8. Lichen substances absent 11

9. Constrictic and stictic acids present; lirellae prominent,
 ± simple with lateral to almost complete thalline margins;
 ascospores muriform, 60–110 µm long *G. vandenboomiana*
9. Stictic acid present; ascospores transversely septate 10
10. Lirellae with apically thick complete thalline margin (*subserpentina*-morph);
 ascospores 70–90 µm long *G. schroederi*
10. Lirellae with thick lateral thalline margin (*marginata*-morph);
 ascospores 45–120 µm long *G. rustica*
11. Ascospores muriform, 20–30 µm long; lirellae erumpent, short to sparsely
 branched with basal thalline margin (*subregularis*-morph) *G. comma*
11. Ascospores transversely septate, ≤50 µm long 12
12. Lirellae with complete thalline margin 13
12. Lirellae with basal to lateral thalline margin 15
13. Unknown lichen substances present; ascospores 3-septate,
 11–17 µm long *G. aptrootiana*
13. No lichen substances present; ascospores 3- or more septate 14
14. Lirellae short and sparsely branched (*negrosina*-morph) *G. negrosina*
14. Lirellae elongate, irregularly branched (*farinulenta*-morph) *G. sitiana*
15. Lirellae short, stellate with basal thalline margin (*geraensis*-morph) *G. dracaenae*
15. Lirellae elongate, irregularly branched with
 thick lateral thalline margin (*marginata*-morph) *G. oxyclada*
16. Proper exciple apically carbonized; hymenium clear 17
16. Proper exciple laterally carbonized; hymenium clear or inspersed 18
17. Lirellae immersed to erumpent, short, sparsely or stellately branched
 (*lineola*- or *coarctata*-morph); ascospores muriform,
 25–45 × 13–20 µm; lichen substances absent *G. pergracilis*
17. Lirellae immersed, ascospores transversely septate,
 24–26 × 4–6 µm; norstictic acid present *G. alboglaucescens*
18. Hymenium inspersed 19
18. Hymenium clear 22
19. Ascospores muriform 20
19. Ascospores transversely septate 21
20. Norstictic acid present; lirellae immersed to erumpent, elongate &
 irregularly branched with thick lateral to complete thalline margin
 (*subserpentina*-morph); ascospores 50–110 × 15–30 µm *G. insulana*
20. Lichen substances absent; lirellae erumpent, short to elongate,
 sparsely to irregularly branched with lateral thalline margin
 (*lineola*- or *deserpens*-morph); ascospores 35–50 × 8–15 µm *G. subvelata*

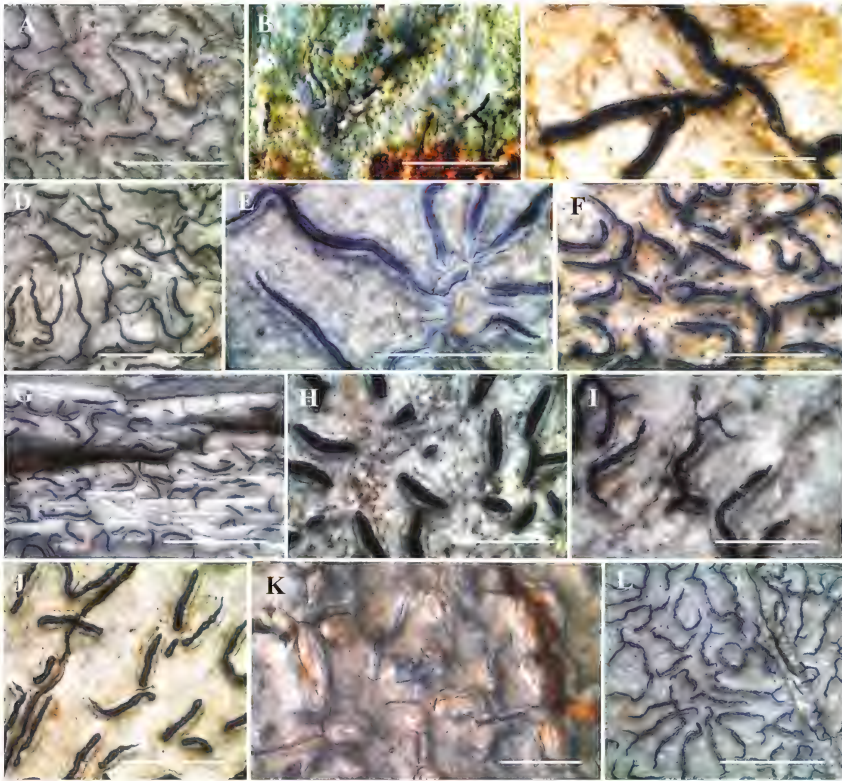


PLATE 1. *Graphis* species from Cameroon. A. *G. ajarekarii*; B. *G. alboglauescens*; C. *G. brahmanensis*; D. *G. daintreensis*; E. *G. exalbata*; F. *G. gloriosensis*; G. *G. gonimica*; H. *G. handelii*; I. *G. immersella*; J. *G. novopalmicola*; K. *G. pseudoaquilonia*; L. *G. supracola*. Scales: A, B, D, F, G, J, L = 10 mm, C, E, H, I, K = 0.5 mm.

- 21. Disc exposed, epruinose (*handelii*-morph); norstictic acid present;
ascospores 5–9-locular, $28\text{--}32 \times 5\text{--}7 \mu\text{m}$ *G. handelii*
- 21. Disc concealed, labia epruinose (*lineola*-morph); no lichen substances;
ascospores 6–10-locular, $20\text{--}40 \times 6\text{--}9 \mu\text{m}$ *G. lineola*
- 22. Ascospores muriform 23
- 22. Ascospores transversely septate 25
- 23. Norstictic acid present; lirellae erumpent, elongate & irregularly
branched with lateral thalline margin (*deserpens*-morph);
ascospores $20\text{--}35 \times 8\text{--}14 \mu\text{m}$ *G. renschiana*
- 23. Lichen substances absent; ascospores more than $50 \mu\text{m}$ long 24

24. Lirellae prominent with basal thalline margin (*hossei*-morph) *G. daintreensis*
24. Lirellae erumpent with lateral to apically thick complete thalline margin
(*subserpentina*-morph) *G. subhianscens*
25. Lichen substances absent; ascospores $\leq 45\ \mu\text{m}$ long 26
25. Lichen substances present; ascospores $\leq 60\ \mu\text{m}$ long 27
26. Thallus partly ecorticate; lirellae thin, flexuous with gently sloping in thalline
margin; labia white pruinose (*caesiella*-morph) *G. furcata*
26. Thallus corticate; lirellae thick; straight or curved with abruptly sloping thalline
margin; labia epruinose (*lineola*- or *deserpens*-morph) *G. pinicola*
27. Norstictic or stictic or protocetraric acids present 28
27. Stictic acid additionally with atranorin or norstictic acid present 31
28. Norstictic acid present 29
28. Norstictic acid absent 30
29. Lirellae erumpent, ascospores $\leq 45\ \mu\text{m}$ long *G. librata*
29. Lirellae immersed to erumpent, short & sparsely branched (*lineola*-morph);
ascospores $45\text{--}60\ \mu\text{m}$ long *G. erythrocardia*
30. Stictic acid present; labia epruinose (*lineola*-morph) *G. immersella*
30. Protocetraric acid present; labia white pruinose (*caesiella*-morph) . . . *G. supracola*
31. Stictic acid & traces of atranorin present;
ascospores 4-locular, $12\text{--}17\ \mu\text{m}$ long *G. tetralocularis*
31. Stictic acid & norstictic acid present;
ascospores 8-10-locular, $35\text{--}55\ \mu\text{m}$ long *G. ajarekarii*
32. Proper exciple completely carbonized 33
32. Proper exciple apically to laterally carbonized; ascospores transversely septate . . 34
33. Ascospores muriform, $30\text{--}65 \times 10\text{--}15\ \mu\text{m}$; lirellae erumpent, elongate & irregularly
branched with lateral thalline margin; disc concealed; labia epruinose
(*tenella*-morph); no lichen substances present *G. plurispora*
33. Ascospores terminally muriform, $50\text{--}80 \times 9\text{--}15\ \mu\text{m}$;
norstictic acid present *G. pseudoaquilonia*
34. Proper exciple apically carbonized 35
34. Proper exciple laterally carbonized 36
35. Norstictic acid present; ascospores $30\text{--}38\ \mu\text{m}$ long *G. exalbata*
35. Lichen substances absent; ascospores $25\text{--}35\ \mu\text{m}$ long *G. glaucescens*
36. Stictic acid present; ascospores $5\text{--}8$ -locular, $25\text{--}30 \times 6\text{--}8\ \mu\text{m}$ *G. brahmanensis*
36. Lichen substances absent 37
37. Lirellae erumpent, short & sparsely branched with lateral thalline margin
(*tenella*-morph); ascospores $15\text{--}45 \times 6\text{--}9\ \mu\text{m}$ *G. tenella*
37. Lirellae prominent, elongate & irregularly branched, lacking or with basal thalline
margin (*striatula*-morph); ascospores $30\text{--}65\ \mu\text{m} \times 7\text{--}12\ \mu\text{m}$ *G. striatula*

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Revision of some *Peziza* collections in herb. TAAM (Tartu)

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ABSTRACT—Forty samples representing the genus *Peziza* kept in the Mycological Herbarium, Tartu, Estonia (TAAM) were studied. Collection data and taxonomic comments are presented.

KEY WORDS—Ascomycota, Pezizales, Pezizaceae

Introduction

This work, part of a series of papers concerning the revision of specimens assigned to the genus *Peziza* Dill. ex Fr. kept in various herbaria, is intended to confirm their importance for the studies on mycological diversity and taxonomy.

Forty samples, initially labelled under various names and located in the Mycological Herbarium, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia (TAAM) (founded in 1950 at the Institute of Biology of the Estonian SSR Academy of Sciences; Parmasto 2011), were studied. Our examinations revealed that not all specimens were originally correctly determined; various specimens were shown to correspond to different species, while others could not be definitely identified. Some of these samples have also been used to carry out molecular analyses, the results of which have been published in previous papers (Medardi et al. 2012, Medardi et al. 2014).

Materials & methods

Morphological and microscopical examinations were carried out on Dried specimens were rehydrated in water or KOH 5% and mounted in water with lactic blue

to highlight spore ornamentation and/or with Melzer's reagent to observe the iodine reaction of the asci. The tissues were examined microscopically with an Optika BK 1301 optical microscope with 40× or 100× (oil immersion) objectives; dimensions were determined from 50 mature spores per collection.

Results

Peziza alcis Harmaja, Karstenia 26(2): 44. 1986.

MATERIAL EXAMINED: ESTONIA: JÕGEVAMAA Co, Kursi, Puurmani Commune, Altnurga, Forestry sq. 95, on moose dung, 8.10.1997, leg. et det. M. Öpik (TAAM 171114, as *P. fimeti*); RÄGAVERE COMM., Uljaste, Lääne-Viru Co., on ground and on dung, in moist *Betula-Picea* forest, s.d., leg. K. Kalamees, det. A. Raitviir (TAAM 71260, as *P. fimeti*). FINLAND: LAPLAND, Kevo, National Park, on moose dung, 28.08.1981, leg. K. Kalamees, det. A. Raitviir (TAAM 122042, as *P. fimeti*).

NOTES: These materials, labeled in the herbarium as *P. fimeti*, have been redetermined as *P. alcis*. The spores were smooth and averaged 15–17(–18) × (7–)7.5–9 µm; the excipulum comprised only one layer (textura globulosa or globulosa-angularis), differentiable into one subhymenium, one medullar excipulum and one ectal excipulum by the cell sizes consistently wider towards the outside of the apothecium. Molecular analysis of TAAM 171114 also confirmed identification of *P. alcis* (Medardi et al. 2012).

TAAM 122042 was judged not to be completely mature, based on the numerous spores still contained in the asci. *Sporormiella australis* (Speg.) S.I. Ahmed & Cain was also noted on the dung.

Peziza arvernensis Roze & Boud., Bull. Soc. Bot. Fr. 26 (Suppl.): LXXVI. 1879.

MATERIAL EXAMINED: DENMARK: ZEALAND, Grib Skov, on ground among stones, 14.08.1965, leg. P. Milan Petersen, det. H. Dissing (TAAM 187941); ESTONIA: TARTU Co., along the road toward Tallinn, in a *Populus* forest, 13.05.2007, leg. L. Tedersoo, det. K. Hansen (TAAM 192338).

NOTES: The spores measured (16–)17–20 × (8–)9–10.5 µm and were ornamented with manifest punctiform warts. A section showed the typical tissues of *P. arvernensis*: subhymenium of textura globulosa-angularis, the medullar excipulum comprising three layers (the upper and lower layers a textura globulosa and the middle a textura intricata), and an ectal excipulum of textura intricata. The habitat of this species is rather variable; that reported for these two collections is within specifications.

Peziza atrospora Fuckel, Fungi Rhenani Exsic.: no. 1224. 1864.

MATERIAL EXAMINED: ESTONIA: TARTU Co., Nõo Comm., Peedu, on ground, 26.07.2000, leg. et det. B. Kullman (TAAM 179346, as *P. pseudovesiculosa* Donadini).

NOTES: TAAM 179346 comprises two specimens, both originally identified as representing *Peziza pseudovesiculosa*. Our redetermination of the species to *P. atrospora* is supported by spores measuring $17\text{--}18 \times 7\text{--}9\text{ }\mu\text{m}$, most with 1–2 oil drops and with elongated warts often connected in short crests $\leq 1\text{ }\mu\text{m}$ high. Other characters include a subhymenium of textura intricata, a medullar excipulum of textura globulosa-angularis with cells $60\text{--}80(110)\text{ }\mu\text{m}$ diam., and an ectal excipulum of textura globulosa-angularis.

Peziza cinatica Pfister, Mycotaxon 8(1): 189. 1979.

MATERIAL EXAMINED: ESTONIA: HARJU Co., Viimsi Comm., Prangli Island, near Idaotsa Farm, on clay soil of a well in *Pinus* forest, 25.08.1960, leg. P. Põldmaa, (TAAM 028962, TAAM 028963, as *P. domiciliana* Cooke).

NOTES: We redetermined this material, bearing the herbarium label *P. domiciliana*, to *P. cinatica*. The ascospores, which measured $11\text{--}13(14) \times (5\text{--})6\text{--}7\text{ }\mu\text{m}$ and contained 2 oil droplets, were ornamented with fine punctiform warts $\leq 1\text{ }\mu\text{m}$. The medullar excipulum is a textura intricata with brownish hyphae (ca. $5\text{ }\mu\text{m}$ diam.) often perpendicular to the hymenium, and the ectal excipulum is a textura globulosa, with some rounded ($20\text{--}50\text{ }\mu\text{m}$ diam.) and elongated ($20\text{--}40 \times 15\text{--}25\text{ }\mu\text{m}$) cells intermixed.

Peziza cinatica is not widely distributed but does not appear to be restricted to one habitat type. Previously reported substrates include ash (Pfister 1979) and burn residue (Dougoud 2001), but the above material (TAAM 028962, TAAM 028963) was collected on clay soil.

Peziza fimeti (Fuckel) E.C. Hansen, Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn 59: 267. 1877 [“1876”].

MATERIAL EXAMINED: RUSSIA: KAMCHATKA, 47 km from Krapivnaya, on dung of *Ursus ursus*, 6.08.1978, leg. B. Kullman (TAAM 187899, TAAM 187900); KRASNODAR, Umpyr, Caucasus Nature Reserve, on dung, 10.08.1976, leg. M. Pallo, det. A. Raitviir (TAAM 064318); Malaya Laba, Caucasus Nature Reserve, on dung, 12.08.1976, leg. M. Pallo, det. A. Raitviir (TAAM 064369); KURIL ISLANDS, Kunashir Island, Lagunnoye, on excrements, 12.08.1970, leg. B. Kullman (TAAM 061513); RESPUBLIKA TYVA, Erzin, on dung, 18.07.1972, leg. B. Kullman (TAAM 065761). TAJIKISTAN. Ramit State Nature Reserve, Mount Hissar, on dung, 12.04.1977, leg. et det. A. Raitviir (TAAM 064569); HOVALING, on bovine excrement, 22. 04.1985, leg. B. Kullman, det.. A. Raitviir (TAAM 115898). UZBEKISTAN: Dshisaki, Turkestan Chain, Kulsai, Zaamini Nature Reserve, in *Juniperus* forest, 24.05.1980, leg. K. Kalamees, det. A. Raitviir (TAAM 199165).

NOTES: Ascospores measuring $(19\text{--})20.5\text{--}22(22.5) \times (10\text{--})11\text{--}12\text{ }\mu\text{m}$ and flesh composed of globose or globose-angulated cells differentiated into a subhymenium, a medullar excipulum (with some filamentous hyphae visible), and an ectal excipulum confirm *Peziza fimeti*.

Our molecular analyses (Medardi et al. 2012) of TAAM 187900 and TAAM 064318 also supported *Peziza fimeti*. *Sporormiella australis* was also found in TAAM 064318. TAAM 115898 is immature.

Noteworthy is the fact that the apothecia TAAM 187899 and TAAM 187900 were found (dung of *Ursus*), a previously unreported substrate.

Peziza howsei Roze & Boud., Bull. Soc. Bot. Fr. 26 (Suppl.): LXXV. 1879.

MATERIAL EXAMINED: ESTONIA: HIJU Co., Hiiesaae Light House, on ground, 18/09/2001, leg L. Vaher, det. B. Kullman ([TAAM 179773, as *Pachyella celtica* (Boud.) Häffner); TARTU Co., Vönnu Comm., Järvelja Forest [sq n. 226 (4)], on ground, 27.09.2001, leg. et det. B. Kullman (TAAM 179788).

NOTES: Both samples were characterized by a subhymenium of textura intricata (hyphae 5–6 µm diam.), a 2-layered medullar excipulum (upper a textura globulosa-angularis with cells ≤25 µm diam.; lower a textura ± intricata toward the upper layer and tending to globulosa-angularis with cells similar to those of the upper layer), and an ectal excipulum of textura globulosa with cells 10–25 µm diam. Ascospores were 17.5–19.5(–22.5) × 8.5–9.5 µm, with fine punctiform warts (0.5–1 µm diam., ≤0.5 µm high), more or less isolated but occasionally joined to form short crests.

Peziza howsei is morphologically similar to *P. emileia* Cooke, but despite several overlapping characters, their different ITS sequences support them as two separate species (Medardi et al. 2014).

Peziza lividula W. Phillips, in Cooke, Mycographia: 161. 1877.

MATERIAL EXAMINED: ESTONIA: PÄRNU Co., Koonga Comm., between Mihkli and Koonga, in *Picea* forest, 16.08.1960, leg. et det. A. Raitviir (TAAM 040951, as *P. howsei*); SAARE Co., Lümanda Comm., Koimia, on ground, 24.08.1960, leg. et det. A. Raitviir (TAAM 041035, as *P. howsei*); HIJU Co., Kärda Comm., next to Kärda, near the road to Käina, in *Corylus* forest, 4.09.1960, leg. et det. A. Raitviir (TAAM 041235, as *P. howsei*); LÄÄNE Co., Hanila Comm., Puhtulaid islet, near Virtsu, in a forest of mixed deciduous trees, 6.09.1960, leg. et det. A. Raitviir (TAAM 041243, as *P. howsei*); TARTU Co., Vönnu Comm., Järvelja forest [sq n. 226 (4)], on ground, 27.09.2001, leg. et det. B. Kullman (TAA 179791, as *P. howsei*). RUSSIA: IRKUTSKAYA OBLAST, Ust-Kutskiy, Erlenga, along a path in a forest, 27.08.1967, leg. M.A. Bondarceva, det. A. Raitviir (TAAM 199080, as *P. howsei*).

NOTES: In this group all samples were at first labelled *P. howsei*. We redetermined them to *P. lividula* based on the following characters: ascospores 15–19 × 8–10(–10.5) µm, each with 2 oil droplets and punctiform warts ± wide (1–1.5 µm diam.), at times more elongate and closed near the extremities where they resemble low skulls; a subhymenium of textura prismatica or prismatica-angularis.

Peziza perparva Harmaja, Karstenia 26(2): 47. 1986.

MATERIAL EXAMINED: RUSSIA: SVERDLOVSKAYA OBLAST, Kytlym, on unidentified dung, 20.07.1973, leg. A. Raitviir (TAAM 062952, as *P. fimeti*).

NOTES: We redetermined TAAM 062952 (labeled as *P. fimeti*) to *P. perparva*. The ascospores measured $(11-12-13 \times 6-7 \mu\text{m})$ and were ornamented with a weak $\pm$ open reticulum; the tissues were arranged differently than those found in *P. fimeti*: subhymenium of textura intricata mixed with a few globose cells, medullar excipulum of textura globulosa or globulosa-angularis, and ectal excipulum with a structure similar to that of the medullar layer but with smaller cells. Also diagnostic of *P. perparva* are the very small apothecia, only 1–3 mm diam.

TAAM 062952, which represents just the second collection of *P. perparva* in Europe (the type collected in Finland and published by Harmaja 1986), expands the range and habitat of this remarkable fungus. Harmaja (1986) stated that *P. perparva* fruits “in moist place on a forest path used by elks, growing in dead plant litter apparently earlier impregnated by elk urine . . .” The Russian specimens appear to grow instead on rodent dung (showing that the fungus is not restricted to elk) at a lower latitude (Lantieri et al. 2011).

Sporormiella australis was also identified on some of the dung associated with the Russian collection.

Peziza varia (Hedw.) Alb. & Schwein. Consp. Fung. Lusat.: 311. 1805.

MATERIAL EXAMINED: ESTONIA: TARTU CO., Tartu Comm., 57 Võru Street, along the border of a woody floor in a cottage, 12.09.1962, leg. et det. A. Raitviir (TAAM 043232, as *P. domiciliana*); near a cement base, 19.12.1977, leg. et det. B. Kullman (TAAM 199053, as *P. domiciliana*); 27-632 Narva Street, on a plastered block of a construction, 20.06.1994, leg. V. Kistanje, det. A. Jakobson (TAAM 199058, as *P. domiciliana*); VILJANDI CO., Kolga-Jaani Comm., Potaste, Vaibla Forest [sq n. 29], Pedja Natural Reserve, on ground, 11.09.1997, leg. et det. B. Kullman (TAAM 157677, 2 collections, as *P. arvernensis*). RUSSIA: KAMCHATKA, 47 km from Krapivnaya, on dung of *Ursus ursus*, 6.08.1978, leg. B. Kullman (TAAM 116381, as *P. fimeti*); YAMALO-NENETSKIY AVTONOMNY OKRUG, Ovgort, on dung of *Ursus ursus* and ground around, 28.08.1976, leg. Murdvee (TAAM 110066, as *P. fimeti*).

NOTES: We redetermined the original identifications of TAAM 116381 and TAAM 110066 (*P. fimeti*); TAAM 157677 (*P. arvernensis*, both specimens); and TAAM 043232, TAAM 199053, and TAAM 199058 (*P. domiciliana*) to *Peziza varia*. We detected a faint superficial roughness on the spores, which measured $(14.5-15-16 \times (7-7.5-8.5(-9) \mu\text{m})$ (smaller than those of *P. fimeti* at $20-22.5 \times 10-12 \mu\text{m}$), and the medullar excipulum showed a median, more or less thin layer of textura intricata, typical of *P. varia*.

Our study of TAAM 116381 and TAAM 110066 revealed that *P. varia* is also able to grow on dung, solving the long-standing question regarding two conflicting interpretations given in *P. fimeti* literature (Medardi et al. 2012).

The excipular structure of *P. varia* overlaps with that of *P. arvernensis* and *P. domiciliana*, which differ from *P. varia* in their larger spores with more or less evident punctiform warts (*P. arvernensis*, $16\text{--}19(-20) \times 9\text{--}10(-11) \mu\text{m}$; *P. domiciliana*, $15\text{--}17(-20) \times 7\text{--}9(-10) \mu\text{m}$, with occasional warts). The builder's rubble habitat of TAAM 043232, TAAM 199053, and TAAM 199058 belongs to the large assortment of substrata on which *P. varia* is able to fruit (Medardi et al. 2012).

Peziza cf. *ampliata*

MATERIAL EXAMINED: RUSSIA: CHELYABINSKAYA OBLAST, Miass, on unidentified dung, 16.08.1973, leg. I. Parmasto (TAAM 062922, as *P. fimeti*).

NOTES: TAAM 062922, bearing the herbarium label of *P. fimeti*, was redetermined as *P. cf. ampliata*. This specimen was previously studied by Hansen et al. (2002). Our molecular analyses group it with *P. ampliata* Pers. (Medardi et al. 2012), although its macro- and microscopic features correspond to those of *P. fimeti*: ascospores measuring $18\text{--}20.5(-21) \times (8.5\text{--})9\text{--}11(-11.5) \mu\text{m}$ and a medullar excipulum comprising large, globose-angulated cells mixed with filamentous hyphae.

Peziza cf. *fimeti*

MATERIAL EXAMINED: ESTONIA: JÕGEVA CO., Puurmani Comm., Alam-Pedja Nature Reserve, on beaver dung, 21.08.1997, leg. A. Kollom, det. A. Raitviir, (TAAM 128087, as *P. fimeti*).

NOTES: This material, labeled as *P. fimeti*, is the “*Peziza* sp. h” analyzed by Hansen et al. (2002), who stated it is phylogenetically different from *P. alcis*, despite its similar microscopic characters: smooth ascospores $15.5\text{--}17 \times (7\text{--})8\text{--}9(-9.5) \mu\text{m}$ and an excipulum consisting of practically only one tissue of (textura globulosa or globulosa-angularis), with three layers differing only in the cell dimensions.

Peziza sp.

MATERIAL EXAMINED: ESTONIA: PÄRNU CO., Koonga Comm., Mihkli Oaks, on ground in oak forest, 16.08.1960, leg. et det. A. Raitviir (TAAM 040944, as *P. howsei*); SAARE CO., Part of “N” Reserve, Viidumäe Natural Reserve, in *Corylus* forest, 26.08.1960, leg. et det. A. Raitviir (TAAM 041077, as *P. howsei*); LÄÄNE CO., Vormsi Comm., Huitbergi, on ground near fir roots, 22.10.2010, leg. et det. B. Kullman (TAAM 190485, as *Pachyella celtica*). RUSSIA: RESPUBLIKA TYVA, Kyzyl, Mazaloeck, on ground, 4.08.1972, leg. A. Raitviir, det. B. Kullman (TAAM 065930, as *Pachyella celtica*); KRASNODARSKIY KRAY,

Guzeripl, on ground, 25.05.1975, Leg. et det. A. Raitviir (TAAM 063473, as *P. howsei*);
 KAMCHATKA, 47 km from Krapivnaya, on dung of *Ursus ursus*, 6.08.1978, leg. B.
 Kullman (TAAM 187898, as *P. fimeti*).

NOTES: Our examinations of these samples did not provide sufficient data for a sure identification, mainly due to the scarcity or lack of needed characters.

Acknowledgments

The authors thank Prof. G. Consiglio and Dr. C. Losi for critically reviewing the manuscript. We want to express a particular gratitude to the late Dr. E. Parmasto, keeper of TAAM (Tartu, Estonia), for his kindness in putting at our disposal the samples and for permission to conduct molecular analysis on some of them, which enabled us to publish our work.

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BOOK REVIEWS AND NOTICES*

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ABSTRACT—Books reviewed include: **GENERAL**—The Outer Spores: Mushrooms of Haida Gwaii (Kroeger & al. 2012); **LICHENS & LICHENIZED FUNGI**—Rare Lichens of Oregon (Exeter, Glade & Loring 2016); Lichens of Uttar Pradesh (Nayaka & Upreti 2013); **ASCOMYCOTA**—Plant pathogenic and endophytic *Botryosphaeriales* known from culture (eds: Phillips & al. 2013); A polyphasic taxonomy of *Daldinia* (*Xylariaceae*) (Stadler & al. 2014); Hypocrealean lineages of industrial and phytopathological importance (eds: Lombard, Groenewald & Crous 2015).

GENERAL

The Outer Spores: Mushrooms of Haida Gwaii.

By Paul Kroeger, Bryce Kendrick, Oluna Česka & Christine Roberts. 2012. Mycologue Publications, Sidney-by-the-Sea BC [www.mycolog.com] in cooperation with the Haida Gwaii Museum (Skidegate BC), Canada. 189 p., ~300 color photos and drawings, paperback. ISBN—978-0-9692237-3-3 Price (excl. postage): \$20 (Canadian) Email: bryce@mycolog.com.



I have been happily immersed in the pages of this gem since last week, which I found temporarily forgotten among the ‘books I must read soon’ shelf. When labors of love are as entertainingly written as this one, the reader definitely benefits, and **THE OUTER SPORES** will charm and edify a great many other mycologists and nature-lovers. The 15 × 23 cm volume covers the mushrooms

* Book reviews or books for consideration for coverage in this column should be sent to the Editor-in-Chief <editor@mycotaxon.com> 6720 NW Skyline, Portland OR 97229 USA.

as well as the history of Haida Gwaii (the post-2010 name for the Queen Charlotte Islands), “a wild and unspoiled corner of the world and the home of the Haida, a people with a deep and rich culture.” This overview of 635 species identified from >2900 mushroom collections gathered during a 5-year survey constitutes the ‘first ... guide dedicated to the fungi of Haida Gwaii.’

The obligatory first chapter “about mushrooms” (here defined as “fungal fruiting bodies large enough to see”) packs an impressive amount of information in ten pages and bears the imprimatur of the indomitable Bryce Kendrick (author of *THE FIFTH KINGDOM*) by answering What are fungi? and What are Mushrooms? before moving on to ascomycete and basidiomycete basics, biological strategies, and a delightful drawing from Oluna’s field notebook. Next follows a brief description of the 150 islands “that comprise a sort of miniature continent” accompanied by a map showing collection sites on the Queen Charlotte Windward Mountains, Skidegate Plateau, and Queen Charlotte Lowlands. A brief cultural history of the indigenous peoples follows; although the Haida did not traditionally consume mushrooms for food, they did venerate Tree-Fungus Man and used bracket fungi for tinder, medicines, and pigments.

Edible mushroom coverage begins with the inevitable safety cautions; here it appears that getting lost in the archipelago is more worrisome than the possibility of death by mushroom: the local forest district and search & rescue crews (motivated by the “significant cumulative cost of numerous searches”) now find it more cost effective to distribute free survival kits (contained within a 1-litre water bottle) to pickers. With much of the area closed to mushroom picking, the authors devote five pages to where, when, and how to collect and eat/or preserve the mushroom quarry, closing with two duplicate (somewhat oddly, given the index on pp. 133–139) lists of the region’s 28 choice edibles with references to later comments and/or photos, the first alphabetized by Latin name and the second by common name. Coverage of the profitable commercial mushroom harvest (primarily of golden, rainbow, blue, and winter chanterelles) precedes a discussion of current and future forest management practices.

Species accounts of 14 edible and 22 “notable” mushrooms, accompanied by generally excellent photos and distribution maps (plus one shot of a dominant herbivore cluster of millipedes munching an *Ganoderma tsugae* in the ‘edible’ mushroom chapter) precede observations on some little known roles and interactions of fungi, such as the truffle-tree-animal triumvirate, sand ecosystems, and nitrogen-seeking *Hebeloma*, *Laccaria*, and *Onygena* ‘corpse finders’. Appropriately enough, next on the menu are toxins, symptoms, and good to somewhat blurry portraits of and maps for the region’s 15 poisonous

mushroom species. Found here is *Inocybe calamistrata* (a mushroom I'd never thought of consuming, but its blue pigmented 'foot' might attract those vainly hoping for a hallucinogenic high). Also portrayed is a mushroom labeled as *Clitocybe* (now the type species of *Ampulloclitocybe*) *clavipes* looking far too slender, small, white, and suspiciously far more like *Clitocybe fragrans*, another known poisonous mushroom. This is one book that stresses the deadly poisonous *Amanita franchetii*—an otherwise beautiful mushroom common throughout Haida Gwaii.

Moving from the deadly poisonous to the strictly hallucinogenic, the next chapter focuses on two "main candidates" for "visionary mushrooms traditionally used as ritual or spiritual intoxicants in Haida Gwaii:" the liberty cap (*Psilocybe semilanceata*, probably introduced by immigrant farmers) and the fly agaric (*Amanita muscaria*, an abundant endemic). Six pages are devoted to the modern natural and legal history surrounding these "magic" mushrooms.

The atlas comprises 139 photos (of variable quality) covering 105 species, including a salal leaf infected by *Valdensia heterodoxa* (which escaped the clutches of the final checklist, although not the index). As this volume is not intended to serve as an identification guide, these photos merely whet the appetite for further information, and the atlas concludes with a list of recommended guides and references.

The authors next summarize the scope of their prodigious five-year inventory efforts: "Over the five years, we made eight field trips to Haida Gwaii of ten to fourteen days. In all, 113 areas were visited ... and we made and preserved 2906 collections representing 635 species and documented 812 species of fungi (not including lichens) from all available sources... We collected on nineteen islands ranging in size of 0.5 hectares to the over 648,000 hectares of Graham Island. The mushrooms ... remained largely unknown until 2003, when we began our survey." The general bibliography precedes six wonderful photos showing the authors (and Oluna's photographer and botanist husband Adolf) in the field.

The final index and acknowledgments follow a 23-page checklist of the 635 species identified thus far from the surveys. Having participated in several long-term fungal surveys (see Norvell & Exeter 2003), I understand all too well how much work was involved in identifying the collections after all the fieldwork was completed. To that end, I do wish the authors had provided a bit more detail on which references they used to identify their finds. In his 2013 book review, Steve Trudell suggested that in the absence of the usual identification caveats (e.g., cf., aff., sensu lato) the identifications should be "taken with a grain of mycological salt." He recently amended this (Trudell 2017) by adding that although the authors had originally included such identification disclaimers, the publisher

unfortunately removed them from the published checklist. Misidentifications or misapplications are to be expected, although the authors' strengths in certain genera (e.g., dark-spored saprobes for Paul, *Inocybe* for Oluna, and *Russula* for Christine) lend more credibility to the checklist. All of us who have conducted similar studies know full well that the real work of identification will continue years after the fieldwork is complete and will applaud the authors for having the courage to publish their results (however preliminary they might be) so promptly and in so engaging a format.

For the most part there are relatively few typographical or spelling errors—the few I noted were '*Beauvaria*' for *Beauveria*, '*Scerotinia*' for *Sclerotinia*, and '*prescottii*' for *prescotii* – and the nomenclature is more or less up-to-date for 2012. I counted three synonym doublets: *Bondarzewia mesenterica* + *B. montana*, *Cystolepiota sistrata* + *Lepiota seminuda*, and *Galerina autumnalis* + *G. venenata* (the last two synonyms of *G. marginata*). Oddly, while '*Lichenomphalia*' is shown on p. 9, only *Omphalina ericetorum* (accepted as *L. umbellifera* after a 25-year nomenclatural battle of epic proportions) appears on the list. Other nomenclatural holdovers include *Boletus* (instead of *Chalciporus*) *piperatus*, *Clitocybe* (instead of *Arrhenia*) *hohensis*, *Coprinus* (instead of *Coprinopsis*) *atramentarius*, *lagopus* & *phaeosporus*, *Gomphus* (instead of *Turbinellus*) *kauffmanii*, *Mycena* (instead of *Roridomyces*) *rorida*, *Omphalina viridis* (instead of *Arrhenia chlorocyanea*), *Paxillus* (instead of *Tapinella*) *panuoides*, *Phlogiotis* (instead of *Guepinia*) *tremelloides*, and *Rozites* (instead of *Cortinarius*) *caperata*. Fortunately, as all names can easily be updated using Index Fungorum, these discrepancies are hardly serious.

All in all, I find *THE OUTER SPORES: MUSHROOMS OF THE HAIDA GWAII* eminently readable, enchanting, and educational and recommend it as an excellent inspiration and guide for other surveys of hitherto untrammelled wildernesses.

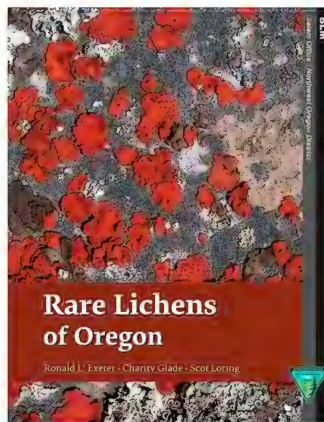
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LICHENS & LICHENIZED FUNGI

Rare Lichens of Oregon. By Ronald Exeter, Charity Glade, Scot Loring. 2016. Government Printing Office, Salem (Northwest Oregon) District, Bureau of Land Management, Oregon, USA. 190 p., ~290 color photos & drawings, hard print + computer disc. ISBN—13: 978-0-9791310-6-6, 10: 0-979-1310-6-5; \$30 (incl. postage, order by phone 1-503-375-5646). Low resolution web version online: <https://www.researchgate.net/publication/309032897>



RARE LICHENS OF OREGON is the fourth lavishly illustrated and impressive identification guide featuring rare, threatened, or endangered species shepherded through the government printing office maze by the first author. Ron, a botanist and colleague who adopted *Ramaria* as a genus needing closer scrutiny during our six-year field studies of ectomycorrhizal fungi, first approached the USDI-Bureau of Land Management Salem District about printing a photographic regional monograph covering a good many “species of interest” cited by the Northwest Forest Plan. *RAMARIA OF THE PACIFIC NORTHWESTERN UNITED STATES* (Exeter & al. 2006), which combined descriptions derived from research papers and personal collections with photographs taken by the original authorities and in the field, proved an immediate success. It was followed two years later by the equally well-received PNW *Phaeocollybia* monograph (Norvell & Exeter 2008) and then eight years later by the outstanding RARE BRYOPHYTES OF OREGON (Exeter & al. 2016).

This valuable addition to Pacific Northwest lichen species literature thoroughly covers 78 special status lichen species cited in the most recent RARE, THREATENED AND ENDANGERED SPECIES OF OREGON (ORBIC 2016) plus *Leptogium compactum* (Stone et al. 2016), a not-yet-listed recently described species currently regarded as rare in Oregon. The 8½” × 11” print copy with sturdy paper cover and glossy pages is an excellent size for displaying the many detailed full color photos and maps.

Acknowledgments, a color bar key to sections, and table of contents precede a brief 2-page introduction, color-coded map of Oregon’s counties and eco-regions, and a 3-page table summarizing in which eco-regions each species has been found. The authors refer readers to MACROLICHENS OF THE PACIFIC NORTHWEST (McCune & Geiser 2009) and LICHENS OF NORTH

AMERICA (Brodo & al. 2001) for detailed lichen structure and biology, keys, and glossaries, which are not included in this volume. The nomenclature has been checked with IndexFungorum (www.indexfungorum.org), and special note is made of nomenclatural changes for four species recently updated in the ORBIC list: *Circinaria* ($\equiv$ *Aspicilia*) *rogeri*, *Gyalolechia* ($\equiv$ *Caloplaca*) *stantonii*, *Pseudocyphellaria hawaiiensis* (= *P. perpetua*), and *Usnea subgracilis* (= *U. schadenbergiana*).

The species descriptions are divided into two sections: 58 macrolichens and 21 microlichens, with the species in each section listed alphabetically, particularly handy given the lack of an index. Left-hand text pages headed by the scientific name face right-hand illustration pages with (1)2–6 illustrations (superbly sharp photos, most taken by coauthor Scot Loring, are occasionally accompanied by lovely and equally detailed pencil habit drawings). Recent synonyms, common names, a field summary, and diagnostic characters precede a brief description of the thallus, apothecia or ascomata, chemistry, ecology, distribution, and comparison with similar species. Each treatment concludes with a list of references, flagging those with color plates. Not surprisingly in a book devoted to rare species, there are ten macrolichen and five microlichen species that are illustrated in color here for the first time.

The volume concludes with seven pages of maps showing distribution by county, a list of abbreviations and symbols, two pages of micro-images of the photobionts *Dictyochloropsis*, *Stichocoocus*, and *Trentepohlia*, a 9-page bibliography, a map of North America, and the CD-disc in a sleeve on a back cover illustrated with six (unfortunately not identified) lovely lichen photos. The inclusion of the algal partner recalled this mycologist's early days of trying in vain to match one lovely 'fungus' (strangely always fruiting on a powdery grayish green 'substrate') with the few *Hymenoscyphus* photos in Breitenbach & Kränzlin (1984). (My gratification at eventually discovering the lichenological nature of *Icmadophila ericetorum* only slightly offset my embarrassment at not recognizing its ubiquitous algal symbiont in the first place.)

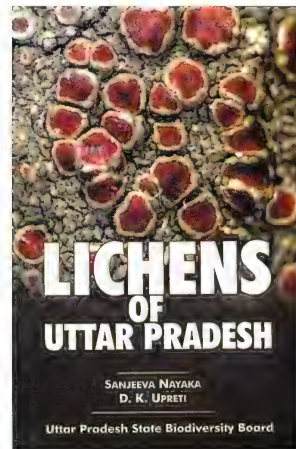
The only drawback to this lovely and welcome addition to Oregon's BLM publishing pantheon is the retirement of its first author as Salem botanist shortly after publication. We wish our man in Salem well, but who now will serve as mycological guardian angel at the Northwest Oregon District Office?

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Lichens of Uttar Pradesh. By Sanjeeva Nayaka & D.K. Upreti. 2014 ("2013"). U.P. State Biodiversity Board (printed by Shivam Arts), Lucknow, UP India. 177 p., ~405 color illustrations. PDF available online: http://www.upsbdb.org/pdf/2014/09/Books/2-Lichens_of_Uttar_Pradesh.pdf.



LICHENS OF UTTAR PRADESH, which arrived by post some time ago, has been buried on my desk patiently awaiting rediscovery. Prepared by two expert lichenologists, this handy-sized (~14 × 22 cm) volume endeavors to expand the lichen coverage of a diminished Uttar Pradesh after the 1999 partition of Uttarakhand state, where until then most Uttar Pradesh collections had been collected. This 2014 publication is based on “critical observation of more than 2250 lichen specimens in NBRI and based on field studies in 35 (out of 75) districts in the state.” Nayaka & Upreti, who treat 135 taxa representing 46 genera in 25 families, contributed 41 new records, thereby adding 61 species (11 new to India) to earlier lists, and excluded 15 cited in Nayaka & Upreti (2011) based on erroneous identification or doubtful occurrence.

Two forwards and an author preface precede a brief but engagingly written introduction that touches on the symbiotic nature of lichens (“at a given time [a] lichen can have members from two to three kingdoms altogether: *Fungi*, *Protista*, and *Monera* sensu Cavalier Smith 2002”) as well as outlines their household uses, medicinal potential, and importance in perfumery and dyes and as air pollution indicators. I was intrigued to learn that lichens form an integral ingredient in Garam Masala (India’s famed powdered spice) and that several different species (35 identified in one spice mixture) may be used in a single spice. Lichens are believed to have curative powers for everything from headaches to leprosy and Uttar Pradeshis use smoke produced by burning *Thammodia vermicularis* to kill germs in milk storage containers.

A brief overview of the geography of the state (home to almost 200 million people) covers the climate, topography, and vegetation. There follow pages on the status of lichen diversity in India, an outline of lichen study in Uttar Pradesh, a note on the current state of knowledge on lichen diversity in the state, a list of the now excluded previously reported 16 lichen taxa, and a conspectus of the 135 taxa covered taxonomically. The introductory pages conclude with methodological notes, a map of Uttar Pradesh districts, and photos of six different lichen habitats.

A 5-page key to the lichen genera of Uttar Pradesh introduces the 139-page taxonomic section, organized alphabetically by genus. Generic names (with authorities and family) are accompanied by formal generic descriptions, numbers of species reported both worldwide and in India, and important literature references. Where needed, a species key precedes individual species treatments, each of which presents a full morphological description, chemistry analysis, and ecological notes accompanied by 3–4 illustrations with photos depicting the lichen macromorphology and/or important anatomical characters plus a district-linked distribution map. Although the photos are small and have a somewhat soft focus, they are definitely adequate for identification purposes, making the book quite useful in the field. References, a welcome 5-page glossary, and a taxonomic index to families and species organized by genus conclude the volume.

Nomenclaturally, the authors have definitely done their homework and have updated names in accordance with Ertz (2009) Lücking (2009), Lücking et al. (2009), Mangold et al. (2009), Sharma et al. (2012), and Staiger (2012); a quick comparison of their names with those cited in IndexFungorum confirms that the nomenclature is current. The care taken to exclude misdetermined (or misreported) species from their 2011 list suggests that their taxonomy is equally reliable. Unfortunately this care does not extend to proof-reading, as there

are a great many typographical or grammatical errors sprinkled throughout the text—a firm ‘fooling’ (for ‘footing’ in the first forward), *Anisomeridium* ‘calicolum’ (p. 22, for *calicolum*, which should, however, be correctly transcribed in Latin as *calicola*), and ‘Moussurie’ (p. 166, for ‘Missouri’) Botanical Gardens are but a few—but fortunately these errors generally do not obscure the meaning and do not detract significantly from the utility of the book.

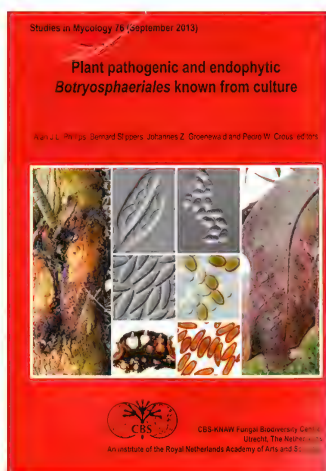
Given the size of the state, it is certain that additional lichen species will be found. The authors are to be commended for providing such a useful resource for continuing lichen research in Uttar Pradesh.

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ASCOMYCOTA

In perusing my bookshelves, I was startled to discover several excellent texts that MYCOTAXON—except for an occasional brief announcement—has pretty much ignored. Because these invaluable STUDIES IN MYCOLOGY volumes (published by the Centraalbureau voor Schimmelcultures) merit much closer scrutiny, I have taken the liberty of briefly summarizing on the next pages the contents of three past issues, all open access and thus immediately accessible online through ScienceDirect.

All papers are accompanied by excellent phylogenetic analyses, superb photographic plates (most tending toward the spectacular), and scientifically rigorous taxonomic descriptions.



Plant pathogenic and endophytic *Botryosphaerales* known from culture.

Alan J.L. Phillips, Bernard Slippers, Johannes Z. Groenewald & Pedro Crous, eds. 2013. STUDIES IN MYCOLOGY 76 (September 2013).

PDFs online:

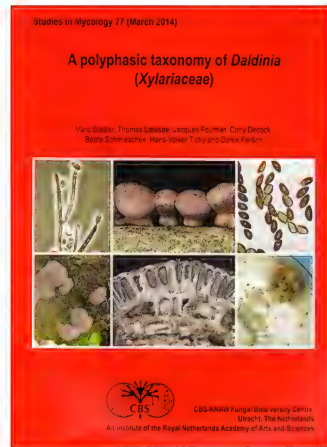
<http://www.sciencedirect.com/science/journal/01660616/76>

This issue, dedicated to Dr. Robert A. Shoemaker, a Canadian plant pathologist well known for his extensive studies on *Dothideomycetes*, presents three papers. “A phylogenetic re-evaluation of *Phyllosticta*” by S Wikee, L Lombard, C Nakashima, K Motohashi, E Chukeatirote, R Cheewangkoon, EKH McKenzie, KD Hyde & PW Crous (pp. 1–29) redefines *Phyllosticta* (noting “in moving to a unit nomenclature for fungi...*Phyllosticta* was chosen over *Guignardia*) and resurrects the older family name *Phyllostictaceae*. A multigene DNA dataset used to investigate 129 *Phyllosticta* isolates (and representing ~170 species names) showed that many were synonyms of *P. capitalensis* and supported 12 new species and two new combinations.

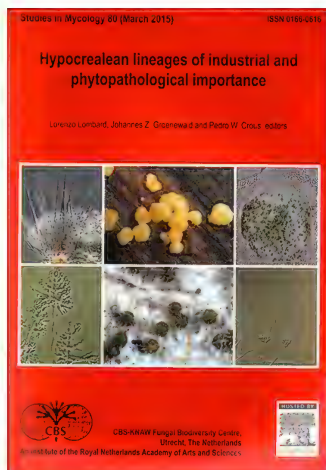
“Phylogenetic lineages in the *Botryosphaerales*: a systematic and evolutionary framework” by B Slippers, E Boissin, AJL Phillips, JZ Groenewald, L Lombard, MJ Wingfield, A Postma, T Burgess & PW Crous (pp. 31–49) covers the phylogenetic evaluation of botryosphaeralean genera known from culture and recognizes six families: the previously named *Botryosphaeriaceae*, *Phyllostictaceae*, and *Planistromellaceae* and the newly proposed *Aplosporellaceae*, *Melanopsaceae*, and *Saccharataceae*.

“The *Botryosphaeriaceae*: genera and species known from culture” by AJL Phillips, A Alves, J Abdollahzadeh, B Slippers, MJ Wingfield, JZ Groenewald & PW Crous (pp. 51–167) focuses on the phylogenetic recognition of 17 genera and 110 species presently known only from culture. Keys to the genera and species are provided, and one new species (*Neofusicoccum batangarum*) and 12 new combinations (in *Botryosphaeria*, *Cophinforma*, *Dothiorella*, *Lasiodiplodia*, *Neoscytalidium*, and *Sphaeropsis*) are proposed.

A polyphasic taxonomy of *Daldinia* (Xylariaceae). By Marc Stadler, Thomas Læssøe, Jacques Fournier, Cony Decock, Beata Schmieschek, Hans-Volker Tichy & Derek Peršoh. 2014. *STUDIES IN MYCOLOGY* 77 (March 2014): 1–143. PDF online: <http://www.sciencedirect.com/science/journal/01660616/77>



For someone only dimly aware that there were more species in *Daldinia* than *D. concentrica*, this thorough exposition of 47 taxa and their morphology, biogeography, chorology, ecology, and secondary metabolites was most welcome. The genus, divided into 5 major groups based on morphological and chemotaxonomic characters and supported by molecular analysis, demonstrates the additional taxonomic value that fungal secondary metabolite profiles can provide beyond the species rank. The genus as amended incorporates many new synonymies, excludes several previously published names, and admits 6 new species and two new combinations to the genus. The rDNA-based phylogeny separates *Daldinia* from *Annulohypoxylon* and *Hypoxylon* while nesting representatives of small predominantly tropical genera within its clade.



Hypocrealean lineages of industrial and phytopathological importance. By Lorenzo Lombard, Johannes Z. Groenewald & Pedro Crous, eds. 2015. *STUDIES IN MYCOLOGY* 80 (March 2015). PDFs online: <http://www.sciencedirect.com/science/journal/01660616/80>

Dedicated to Amy Rossman, an American mycologist “well known to mycologists and plant pathologists around the world for her scientific passion for ... hypocrealean fungi”

[not to mention her engagingly cheerful and encouraging personality], these five papers focus on the taxonomy and revision of the generic and species concepts in the *Hypocreaceae* and *Nectriaceae* (*Hypocreales*).

“Biodiversity of *Trichoderma* (*Hypocreaceae*) in southern Europe and Macaronesia” by WM Jaklitsch & H Voglmayr (pp. 1–87) recognizes 90 *Trichoderma* species (the 17 new to science dazzlingly illustrated by excellent photos) supported by *TEF1*-, *RPB2*-, and *ACL1*-based sequence analyses from >650 identified specimens.

“Diversity and potential impact of *Calonectria* species in *Eucalyptus* plantations in Brazil” by RF Alfenas, L Lombard, OL Pereira, AC Alfenas, and PW Crous (pp. 88–130) identifies 20 *Calonectria* species new to science plus *C. pseudopteridis* (nom. nov. for *Cylindrocladium macrosporum*) based on sequence analyses of the β -tubulin, *CMD*, *TEF1 α* , and *HIS3* DNA regions and reveals the *C. pteridis* complex as the most common species complex in Brazil’s *Eucalyptus* plantations.

“Novel taxa in the *Fusarium fujikuroi* species complex from *Pinus* spp.” by DA Herron, MJ Wingfield, BD Wingfield, CA Rodas, S Marincowitz & ET Steenkamp (pp. 131–150) explores the diversity of *Fusarium* species associated with four species of diseased pine in Colombian plantations and nurseries. β -tubulin and *TEF1 α* sequence analyses of 57 isolates sampled from plants displaying stem cankers, branch dieback, and seedling root/collar rot identified more than ten *Fusarium* species, of which five are named as new. Four of the new species displayed a level of pathogenicity on *Pinus patula* comparable to that of the pitch canker pathogen *F. circinatum*.

“New species, hyper-diversity and potential importance of *Calonectria* spp. from *Eucalyptus* in South China” by L Lombard, SF Chen, X Mou, CD Zhou, PW Crous & MJ Wingfield (pp. 151–188) summarizes surveys of *Eucalyptus* displaying symptoms of leaf blight in Guangdong, Guangxi, and Hainan provinces that produced a large number of *Calonectria* isolates. Of the 21 isolates identified using the Consolidated Species Concept employing morphological characters and DNA sequence comparisons of the β -tubulin, *CMD*, *TEF1 α* , and *HIS3* gene regions, 18 represented new taxa (12 in the Sphaero-Naviculate Group; 6 in the Prolate Group). Southeast Asia appears to represent a centre of biodiversity for the Sphaero-Naviculate Group, which could be an important constraint to eucalypt forestry in China.

“Generic concepts in *Nectriaceae*” by L Lombard, NA van der Merwe, JZ Groenewald & PW Crous (pp. 189–245). Nectriaceous species are unified by phenotypic characters such as uniloculate ascomata that are yellow or orange-

red to purple and exhibit phialidic asexual morphs. The family's generic concepts have been poorly defined due to the unavailability of DNA sequence data for many genera. A multi-gene phylogenetic analysis of known nectriaceous genera using partial 28S LSU sequences and the ITS1+5.8S+ITS2, ACL1, RPB1, RPB2, ACT, TUB2, CMDA, HIS3, & TEF1 regions from available type and authentic strains support six new genera (*Aquanectria*, *Bisifusarium*, *Coccinonectria*, *Paracremonium*, *Rectifusarium*, *Xenoacremonium*) that accommodate species previously classified based solely on morphology. Additionally several generic names are proposed for synonymy based on the abolishment of dual nomenclature, and a new family (*Tilachilidiaceae*) is introduced for two genera previously accommodated in the *Nectriaceae*.

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NOMENCLATURAL NOVELTIES AND TYPIFICATIONS

PROPOSED IN MYCOTAXON 131(4)

- Abieticola* Hyang B. Lee [MB 811702], p. 755
Abieticola koreana Hyang B. Lee [MB 811703], p. 755
Alternaria parvicaespitosa Gannibal & D.P. Lawr. [MB 816666], p. 784
Apiosordaria hamata B. Wu, K.D. Hyde, Jing Z. Sun & Xing Z. Liu
[MB 812280], p. 852
Arthromoniliphora S.S. Silva, Gusmão & R.F. Castañeda
[MB 813427], p. 822
Arthromoniliphora araucariae S.S. Silva, Gusmão & R.F. Castañeda
[MB 813428], p. 822
Blodgettia saprophytica C.L. Yang, Z. Li & X.G. Zhang [MB 819496], p. 909
Discopycnothyrium Hongsanan & K.D. Hyde [IF 551024], p. 862
Discopycnothyrium palmae Hongsanan & K.D. Hyde [IF 551023], p. 863
Entalbotroma J.D. Rogers & P.R. Johnst. [MB 817225], p. 766
Entalbotroma erumpens J.D. Rogers & P.R. Johnst. [MB 817226], p. 768
Nawawia oviformis J. Peng & Z.F. Yu [MB 815592], p. 737
Pezicula chiangraiensis Ekanayaka & K.D. Hyde, [IF 551789], p. 744
Phaeocollybia pakistanica J. Khan, Sher & Khalid [MB 818193], p. 892
Podosporiopsis Jian Ma, X.G. Zhang & R.F. Castañeda [IF 551862], p. 774
Podosporiopsis obclavata Jian Ma, X.G. Zhang & R.F. Castañeda
[IF 551864], p. 775
Podosporiopsis sinensis Jian Ma, X.G. Zhang & R.F. Castañeda
[IF 551863], p. 774
Podosporium bacilliforme J.Y. Wang, Yu M. Cai & X.G. Zhang
[MB 819468], p. 842
Strigula sinoaustralis S.H. Jiang, X.L. Wei & J.C. Wei [FN 570267], p. 797
Thelenella haradae J. Halda, Xin Y. Wang, J.A. Ryu & Hur [MB 819398], p. 806
Zasmidium oleae Y.L. Guo, X.W. Xie & B.J. Li [FN 570159], p. 791

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Richard Paul Korf (1925–2016): A Celebration

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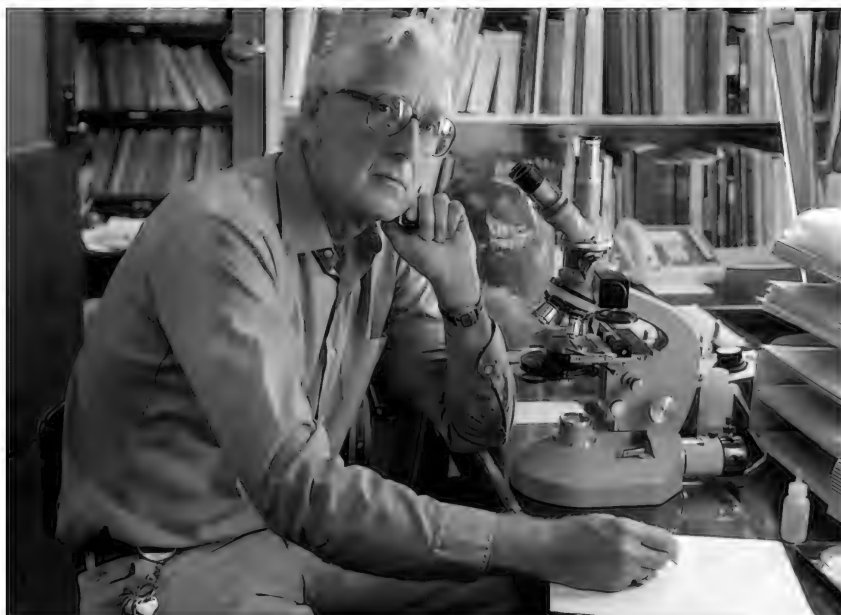
ITHACA JOURNAL, DECEMBER 9, 2016—“Richard Paul Korf, age 91, died August 20, 2016, at home in Ithaca, NY, leaving behind his wife, Kumi, with whom he was enchanted for over 50 years, and his adored children Noni, Mia, Ian, and Mario, and grandchildren Maïa Vidal, Zak Korf, Zephyr Gettelman, Mason Korf, and Matéa LeBeau. Dick’s academic life began at Cornell University in 1941 at the age of 16, and he received his Ph.D. in Mycology from the Department of Plant Pathology in 1950. He taught one year in the Botany Department of Glasgow University (Scotland) before returning to his alma mater where he continued in his teaching and research until his retirement as an Emeritus Professor in 1992. Dick was elected President of the Mycological Society of America, received two Fulbright awards (Japan and Belgium) and an NSF Senior Postdoctoral Fellowship (Japan). He was offered but declined a Guggenheim Fellowship, and has received several honorary awards for his teaching and research.”

ABSTRACT—With this section, MYCOTAXON celebrates the life of its mentor and co-founder, Richard Korf, who died after a brief illness at home in 2016 surrounded by his family and faithful companion, Meena. Materials from other publications and web posts accompany reminiscences by Dick’s colleagues, students, authors, and family.

PREFACE: Eliciting contributions from friends and colleagues after any death is difficult, but arranging them into a coherent whole in a scholarly research journal established by the ‘honoree’ himself has proved doubly so. Those who knew Prof. Korf as ‘Dick’ submitted fond memories that amuse, inspire, and make readers wish they had known him better. His many contributions were so varied that we have chosen to cite only a few. Dick would undoubtedly be gratified that this collection of reminiscences is published more or less as submitted and forgive us for allowing his full accomplishments to be enumerated elsewhere; he would, however, heartily disapprove of my shameful use of material previously published or blogged elsewhere.

A complete scholarly obituary should include all publications written, offices held, organizations joined, and end with one sentence dispensing of family relationships. However, Korf’s papers and books are listed on www.MYCOTAXON.com/pubsRPK.html and the Richard P. Korf Wikipedia entry cites three genera and 16 species named after

him and outlines his formidable curriculum vitae, and so we opt instead to celebrate his life with an eclectic mixture of photos and fond reminiscences. Mourning the passing of our self-professed curmudgeon and in the spirit (if not the words) of Shakespeare's Marc Anthony, we all have come here to praise him.



K.E. Loeffler; courtesy of the CUP image collection

FIG. 1—Dick at his microscope in his Cornell office in 1985

Mycotaxon remembers

Not surprisingly, I first became acquainted with Dick through the colorful MYCOTAXON volumes on the Oregon Mycological Society library shelves. Informed by 1979 OMS Foray guest mycologist Harry Thiers that we needed a species checklist, I had begun compiling a master list of Pacific Northwest macrofungi from journals, books, and reprints. The tedious page-by-page scrutiny of MYCOTAXON prompted me to write to its Editor seeking some sort of index. By return mail a manila envelope arrived from a Dr. Richard P. Korf filled with a bundle of typed sheets covering the first 8 volumes accompanied by request for a quick return of this, his only copy, as he soon intended to publish a cumulative index (i.e., Korf & Gruff 1985). Researchers today—with immediate access to INDEX FUNGORUM, MYCOBANK, and the Internet—cannot appreciate the true value of what Dick so generously shared with a complete stranger whose only credential was an interest in fungi. It did, however, net him an appreciative individual subscriber.

I met Professor Korf in person in 1990 as a first-year graduate student. My supervisor, Joe Ammirati, dispatched me to escort our esteemed visitor across the University of Washington campus to the Suzzallo Library. Praising the MYCOTAXON submission procedure, I thanked him again for his 1980 generosity; he in turn proudly spoke to MYCOTAXON's success (despite dire predictions to the contrary) as a journal prepared by authors mentored by their chosen peer reviewers. We agreed that open peer review was peculiarly suited to taxonomy where the ultimate peer is the scientist who must test post-publication hypotheses and that delays inherent in journal-controlled blind reviews were unnecessary. (Needless to say, three years later my first research papers were published in MYCOTAXON. Dick's acceptance letters, generally concluding with a kindly "Thank you for your fine contribution" were the source of much preening on my part.)

The 1991 Mycological Society of America meeting (my first) in San Antonio underscored the extent of Dr. Korf's prodigious mycological contributions. We chatted briefly before the awards ceremony where he received the Distinguished Mycologist award from a society that he had served as Councilor (1958–1960, 1965–1968), Secretary-Treasurer [and MSA Newsletter editor] (1969–1970), Vice-President, and President (1970–1971). Not confined to one continent, Dick



Nomi Korf

FIGS 2 & 3—Fathers of Mycology:

FIG. 2. Elias Magnus Fries (1794–1878), sometime after his 80th birthday. FIG.3. Richard 'Elias Fries' Korf (1925–2016), aged 69 in full Friesian regalia for a performance as Elias Fries at Cornell.



Chandler Photographers; Courtesy Amy Rossman

FIG. 4—Dick Korf chatting with ‘Case’ Bas at the First International Mycological Congress at Exeter, UK, in 1971.



Amy Rossman

FIG. 5—Back in Eliasian fieldgear, Dick reprises his performance at Oslo’s seventh International Mycological Congress in 2002 and gives a big hug to Karen Hansen.

had also served on the International Botanical Congress (IBC) Nomenclature Section's Committee for Fungi & Lichens and was 1973–1977 chair of the International Mycological Congress's Standing Nomenclature Committee. An international force to be reckoned with, Dick received the Ainsworth Medal, the International Mycological Association's highest award, in 2010.

Back in 1994, I waltzed up to his 200-year-old Friesian persona at IMCV in Vancouver and, in an ill-advised attempt at humor, 'complimented' him with, "you really don't look as OLD as you are!" Immediately drawing himself up, he glared haughtily at me. I'm still not certain whether he didn't 'get' it or merely remained steadfastly in character. Whatever his true feelings, I promptly wilted and scurried away, abashed.

Despite his self-proclaimed status as curmudgeon, Dick (whom in 1998 I still addressed somewhat timorously as 'Dr. Korf') offered a sympathetic pair of eyes and amusing advice during my years as INOCULUM Editor and MSA Secretary. On the heels of my second MSA newsletter, I received a cordial note with the news that Heinz Cléménçon was associated with the University of



FIG. 6—The infamous "oh no, another erratum" cartoon shared by Dick

Lausanne (not Lucerne) accompanied by a small cartoon to soften his proof that I was not perfect. This goaded me to establish an 'Embarrassing omissions, additions, and corrections' section, printed far too often to suit either of us. He followed the cartoon with later gems: [August 1998] "Nothing new to add, but on page 11 I learned that in 1988 Scot Rogers became a Department of Botany at the University of Washington. I'll bet he was surprized [sic]!" and [October 1998] "Dr. Richard Hanlin's proper first name is "Dick (as in Hanlin), not Robert (as in Heinlein)." My pleasure at his correctly kenning the source of my temporary confusion re Dick Hanlin did not, however, lessen my chagrin at misplacing the name of so amiable an MSA former president and friend.

'Dr.' Korf's phone call in 2003 asking me to serve as next MYCOTAXON Editor-in-Chief left me flabbergasted. Thoroughly tired of deadlines, errata, and other editorial nightmares, I had placed editorships behind me. Despite the small honorarium, however, Dick's offer was too professionally tempting to refuse. It would be fun, I mused, to cobble together something for a change without having to write anything (ignoring former Editor-in-Chief Jean Boise Cargill's dry warning that Don Pfister's estimate of one day per week for her editorial work fell short of the mark). I mused wrongly, of course, but accepted Dick's offer and prepared by ordering my last few back MYCOTAXON volumes.



FIG. 7—Grégoire Hennebert and Dick Korf plot out their new journal, MYCOTAXON, at the Université catholique de Louvain (Belgium) in 1973.

MYCOTAXON was Dick's mycological love child. In his farewell to co-founder Grégoire Hennebert, who retired from our masthead after 32 years as French Language Editor (including 17 years as Book Review Editor), Dick proudly recapitulated the founding and evolution of their journal even as he worried over the economic feasibility of printed taxonomic publications (Korf 2007).

From its 1973 inception to 2016's thwarted free open access, the journal remained a source of joy and worry for its Cornell co-founder. After 'retiring' from active duty as 1974–1991 Managing & English Language Editor, Dick used the title Editor-in-Chief for his appointed successors—Jean Boise-Cargill (1991–1998),

Pavel Lizoň (1998–2003), and me (2004–present)—who ostensibly handled the day-to-day production side. Dick nonetheless held onto the reins as chief Journal Preparation wrangler (1991–2004), Business & Subscriptions Manager (1991–2006), and MYCOTAXON Ltd. Treasurer (1999–2015).

A surprisingly patient Dick projected two volumes of two 250-page issues for 2004 before returning in 2005 to four 500+-page volumes. That first year we imposed a cohesive MYCOTAXON style upon the previous assortment of differing font sizes and styles and moved from print-ready pages and reviews mailed in by authors to computer-prepared files submitted by email. Although prone to fretting about overworking his all-too-willing new E-i-C, Dick was mightily pleased that we managed the changeover by the end of 2004, although we both despaired of making my overly complicated 'Instructions to Authors' easily understood by foreign authors and word-processing neophytes.

Dick granted his E-i-C complete freedom but did wistfully ask that we retain his journal's title and size. He needn't have worried. The name MYCOTAXON is sacrosanct, and although a book format may appear outdated for hard copy, its smaller page size permits readers to scan a complete page on-screen without having to scroll up and down moving from column to column. (We did have a bit of a 'discussion' over my 2013 desire to shift the titles and sub-titles left from page center; but after checking other journal formats, he somewhat grumpily acquiesced.)

Nonetheless, when his new E-i-C changed the unwieldy subtitle—AN international journal for research on taxonomy & nomenclature of fungi, including lichens—to “THE international journal of fungal taxonomy and nomenclature,” Dick was ‘thrilled’ that Grégoire’s and his journal now rightfully merited the word ‘THE.’ He fretted that lichenologists might feel excluded until I pointed out that ‘fungal’ democratically covered symbionts, myxomycetes, and other fellow travelers.

Dick definitely encouraged my nabbing Shaun Pennycook as Nomenclature Editor in 2005 when I realized that adding nomenclatural research to author and reviewer correspondence, manuscript revision, and converting separate text and illustration files into PDFs was too much for one person. Shaun’s volunteering to take over manuscript accession proved an unexpected boon and soon neither of us could envision the journal without him.

Dick was passionate in his desire that MYCOTAXON survive without page-charges through subscriptions alone. [Whenever I asked whether MYCOTAXON showed a profit, Dick would respond, “Occasionally,” and then change the subject by telling me not to concern myself with financial matters.] It had long been obvious that rising postal rates would soon preclude our mailing heavy print volumes to subscribers. However, after he paid out of pocket what I considered an enormous sum to keep the journal solvent for one year, even Dick realized that our financial model had to change. Aware that the next International Code would permit publication of names online (see Norvell 2011), Dick urged the editorial advisory board and owners to move to online publication in 2011. They agreed.

Unfortunately, although less expensive than hard copy, online publication is not free. Webmaster and daughter Noni found an acceptable online host (Ingenta), but as subscriber fees still could not cover journal costs, Dick and Bookkeeper Hannes Maddens agreed to institute page charges. A highly distressed Dick, who had hoped to offer a completely open-access journal, discovered that obligatory open access fees were too high for many long-time authors. Dick was bitterly unhappy that a completely open access MYCOTAXON was economically unfeasible in 2011.

Naturally two strong-willed editorial types did disagree on occasion, and there were definitely times when I felt the founder’s axe poised dangerously close to my neck—usually when a quarterly deadline passed without a publication. MYCOTAXON was founded on a prompt turn-around. Our moves to editorially prepared copy and rigorous scientific and nomenclatural editorial review produced countless delays. Predictably submissions lagged, due also to our newly instituted page charges, competition from an increasing number of online mycological journals, and in 2012 a “perfect storm” of editorial illnesses.

Our 500-page target proved increasingly unattainable; Dick felt that anything under 300 pages did not merit the term ‘volume.’ We eventually resolved this unhappy situation by changing ‘volume’ to refer to the annual publication of four quarterly issues delivered more or less on time. [It is sadly ironic that this paper has delayed publication of the final issue of volume 131.]

Fortunately such collisions were few, and I always looked forward to our weekly chats, generally launched with neutral weather and health reports before moving on to thornier problems, such as the discovery that for seven volumes Else Vellinga had been identified as “**BOOL REVIEW EDITOR**” on the masthead, and no one had noticed.

Dick also dispatched frequent pep-talks. To banish my doldrums over author errors, he wrote, “We started with the concept that errors were the fault of authors, not ours as editors/publishers. Any paper that requires editorial correction by you (as opposed to corrections suggested by reviewers) should be rejected outright until authors (or their reviewers) make their corrections. If the title is incorrectly punctuated or misleading or non-informative, I’d reject the paper outright till they get it right ... Your health is more important than always trying to be the best friend of folks who don’t deserve your pains! Curmudgeonly, and simultaneously compassionately, Dick.”

When after too many years of stomach-crunching deadline madness, he buttressed my decision (to correct only PDFs with editorial errors but not author errors suddenly discovered by the authors after conversion) with, “Dear over-worked E-i-C, Good idea. I think all author errors should appear in the errata; we will change only editorial errors in PDFs before publication. Their submission is what we print... Anything that will relieve your stress and Shaun’s is an improvement. I also highly applaud a much stricter rejection option. Authors and reviewers both need to be made aware of their shortcomings. Fondly, Dick.”

In 2011, amused by his body’s commitment to fungi after the successful removal of a nasal melanoma, Dick wrote, “One bright yellow “mushroom” almost as big as a golf ball (but flatter) remains, holding the skin graft from my neck area in place while the blood vessels grow up into it and my nose becomes grafted correctly. Mushroom probably has to be there a week or so. Think I’ll stay close to home for the next few days. Dick”

Ever the optimist, Dick always looked forward, as witness his response to his co-founder’s condolences on our move to online publication. Grégoire had written, “I do not know who chose the cover color [black] of the last printed issue of MYCOTAXON after 36 years of printed life. For me it expresses adequately my feeling receiving the last physical volume of your child. ...Now after its physical

death, its soul will continue a virtual life, only easily accessible and usable to illuminated minds.” Dick responded, “The color choice is always Lorelei’s. Her feelings were like yours about the death of the printed journal. I did not agree. I would have preferred some verdant GREEN or STARBURST to celebrate the journal being reborn in a less environmentally destructive format.”

Richard Paul Korf was golden. Not to mention immortal, as noted by his former student and friend, Wen-Ying Zhuang: “Dick left us, but his contribution to mycology will stay forever.”

Unfortunately, that doesn’t mean we don’t miss him—fiercely.

—LORELEI NORVELL

MYCOTAXON Editor-in-Chief, Portland, OR



FIG. 8 (LEFT)—Meredith Blackwell, H. Peter Kahn, and Don Pfister with Dick during a 1994 Cornell Hoot at the Ringwood Preserve, Cornell Botanic Gardens. FIG. 9 (RIGHT)—Former MSA presidents Robert W. Lichtwardt (1971–1972) and Richard P. Korf (1970–1971) with President Orson K. Miller, Jr., and Kumi Korf at the 2000 MSA annual meeting in Burlington, VT.

“I was sad when I heard that Dick passed away. Dick meant a lot to me. He was like a grandfather, mentor, and close friend. Even though I’m sad that he isn’t around anymore, I will always have lots of happy memories of all of the great things he has done.

“I know I will never forget Dick. As I’m typing this at my desk, there are reminders of him everywhere I look. I’m typing this on my MacBook, which Dick got me hooked on after giving me his old Macintosh about 10 years ago. On top of the stack of mostly MYCOTAXON-related mail on my desk, is a bank statement for one of my first bank accounts that Dick helped me set up. When I look out of my window at my driveway, I see the forsythia hedge that Dick helped to pay as a housewarming gift when I bought my house. In the kitchen is the microwave he bought for my girlfriend and me when we first moved in together. On the kitchen counter I see bottles of Guinness, one of my favorite beers that I first tasted during one of the many times Dick took me out to eat. Hanging on our coat rack is my favorite old fishing hat that Dick gave me about 15 years ago. I still think of him whenever I wear it.

“These little reminders bring back lots of happy memories about good conversations and stories during long car rides, fishing and swimming, late night card games, and the many delicious dinners (like fish head soup) that Dick prepared. Besides happy memories, all these little reminders of him also bring back memories of all the good things I have seen him do over the years.

“I’m not going to spend hours writing about all of his kind and charitable acts towards me and other people. I’m assuming most of his students, colleagues, and friends must have experienced his kindness themselves. I just want to share Dick’s greatest gift to me.

“Dick’s greatest gift to me was his confidence in me. Dick’s trust and confidence in me has given me so much self-confidence. Dick believed I would be successful in life, and he was always looking for ways that he could support me. He always encouraged me to continue my education, but when I wanted to work as soon as I graduated high school instead, he helped connect me with people who could give me a job. Maybe he somehow knew I would attend college the next year. When I went on my first date with my girlfriend, he let me use his Miata sports car. When I told him I wanted to try living in a cabin in the woods like Thoreau, he didn’t tell me I was crazy. Instead he sold me a small piece of land he owned in Dryden. And, what I am most proud of is that he trusted me to help run the journal MYCOTAXON that meant so much to him. I think Dick’s generosity and kindness towards so many came from the fact that he believed in the goodness of people the same way he believed in me.

“I like to think that the kindness that Dick showed me lives on in me. Dick once gave me a gift membership to the American Civil Liberties Union (a charity I had never heard of), and now I am proud to support this and other charities that he introduced me to. At work, I enjoy giving students confidence that may help them get through school, just like Dick did for me.

“Like Dick, I am also trying to help MYCOTAXON become an open access journal, or if that is not possible, I would like go back to allowing taxonomists to publish manuscripts quickly for free. Dick and I spent lots of time trying to decide whether to continue to allow authors to publish for free (this was how Dick ran the journal) or charging authors so that we could make the journal free to everyone.

“Even though Dick isn’t here now, his memories, his generosity, and his many kind acts will always be with us.”

— HANNES MADDENS

MYCOTAXON Bookkeeper & Subscriptions Manager, Ithaca NY

The Theatre

Anyone acquainted with Dick was fully aware of his theatricality, but they might not have known of his real acting chops. Regarding this extra-mycological passion, friend and former MYCOTAXON E-i-C Pavel noted (Lizoň 2016), “Dick was performing as an amateur actor both on stage and in the recording studio, and for few years he even served as the Chair of the Department of Theatre Arts at Cornell.”

Dick himself explained, “My acting career began at an early age in Riverdale Country School in New York City, eventually being cast in major roles in three annual outdoor productions of Shakespeare’s plays. ...The theatre has been my lifelong passion. I performed during my college years at Cornell University (where I later became a professor) and I continued, both on stage and in radio dramas. While on my final sabbatical leave before retirement I took a fling at off-off-Broadway performances of three plays while in New York City.” (Korf 2006)

EZRA, Cornell’s quarterly magazine, revisited Dick’s participation in the 1946 production of a play on the founding of the college (Wilensky 2013): “Richard Korf ‘46, Ph.D. ‘50, Cornell professor emeritus of mycology, played ‘Angus Dangit’ in ‘Once Upon a Hill’ (one of his lines: ‘Humph. Makin’ a school outa Ezry’s farm!’).

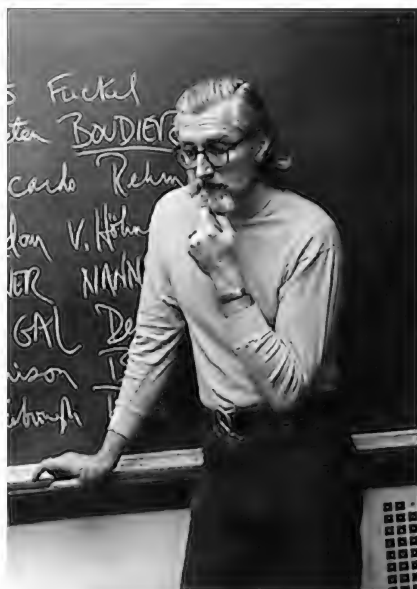


FIG. 10—Dick’s 1980 actor’s promotional photo



FIG.11—The soon-to-be illustrious Professor Korf as a hayseed peeks out at the audience (extreme stage right, second from bottom) in a 1946 Cornell curtain call.

“It was just a fun thing to be in,’ he recalls, noting that his dog also got to be in the show, wearing a Cornell sweater. He also remembers that there was a spittoon sound effect used off stage, which was ‘used to emphasize some of our comments.”



Howard H. Lyon; courtesy of the CUP image collection

FIG. 12—Professor Korf at the blackboard of Plant Science 326 lab in 1972.

Korf at Cornell

Amy Rossman and Wen-Ying Zhuang (2016) recall Dick’s introduction to his alma mater and mycology. “Having entered Cornell University intending to become a gentleman farmer of Cornish game hens (as I remember it), he soon switched to mycology under the influence of Harry M. Fitzpatrick (1886–1949), completing his PhD in 1950. With the untimely death of his major professor, Dick joined the faculty of the Department of Plant Pathology at a relatively young age. He was thus the major professor for several students who were about his age or older. He started as Assistant Professor in 1951, and eventually served as full Professor of Mycology [at Cornell] from 1961–1992.”

In his 90th birthday wishes, Pavel (Lizoň 2016) rightly referred to Cornell as Dick’s life-long companion. “Under his guidance, more than 20 PhD students graduated (Korf 1991), the first three—Robert A. Shoemaker, Robert L. Shaffer, and Martin A. Rosinski—already in 1955.”

The Cornell Hoot

“When hunting mushrooms, it’s easy to lose students in the woods. That’s why we practice the Cornell Hoot. Learning the Hoot is a highlight of the first Fall field trip. With students gathered round, I describe the exquisite art of collecting mushrooms, hand out crisp Cornell apples and maps, and demonstrate the Cornell Hoot: a rising “Ah-OOOT!” My students shuffle uncomfortably, but soon they can’t help but smile. Now we practice together Ah-OOT! Ah-OOOOT! Ah-OOOOOT! Even the shy ones can’t resist it. We do it again in unison, very loudly. A distinctive sound.



FIG. 13—Dick and field companions after a 1994 Cornell ‘hoot’ at the Ringwood Preserve, Cornell Botanic Gardens.

“I’ve wondered where the Cornell Hoot came from. I learned it from my predecessor, Dick Korf. Imagine my tingling excitement when I encountered this passage in the 1903 travel diary of my great-great predecessor at Cornell, George Atkinson, who was visiting the Botanical Gardens at Kew, in England: “As time for closing the gates came on I heard musical voices from different parts of the garden sing “all out, all out.” A custom very old, and now it is such a perfunctory call that one can scarcely distinguish the words. It often sounds more like ‘Ah-laio.’

“...Then I heard back from Dick Korf. He said ‘Oh, it’s the Cornell HOOT now, is it?’ And he told me it was ENTIRELY HIS OWN INVENTION. Recent and local, not at all what I was thinking! It made me laugh out loud. But an enchanting story still. Here’s his tale: ‘It was when I was at Ringwood with one of my first class field trips, maybe in 1951, and noticed that all students had not returned. I thought it to be the loudest and most distinctive calls in my vocal repertoire. I am pretty sure it didn’t come from one of the many plays I did as an undergraduate and graduate student, learning to project my voice, even a whisper, to reach the back row in the theatre, which Professor Alexander Magnus Drummond demanded of us.’”

— KATHIE T. HODGE, Director
Cornell Plant Pathology Herbarium, Ithaca NY



FIG. 14—Dick chats with MYCOTAXON E-i-C Pavel during the 1994 hoot.

CUP—Cornell Plant Pathology Herbarium

Four days before my usually scheduled phone call to Dick, I learned of his sudden demise from Scott LaGreca, who wrote, “We are sad to share that Richard P. Korf passed away yesterday, at the age of 91. There was a small reception at Noni’s house last night in Ithaca and I’ve never seen such an upbeat group, celebrating a person’s life. It was heartwarming,” Scott later added, “At a reception Saturday night in Ithaca, his remarkable family and friends raised toasts of Guinness (his drink of choice); remembered his generosity, humor, and wisdom; and sang his favorite songs.” Below is Scott’s formal contribution to our celebration of Dick’s life.

Dick’s many personal and professional connections have made our herbarium (CUP) what it is today. Dick contributed thousands of specimens from around the world to our collection. I work every day surrounded by those specimens; they are part of Dick Korf’s legacy: thousands of specimens, as well as his notebooks and correspondence. Those specimens have people’s names and places on them, which tell Dick’s entire story.

My very first herbarium specimen (LaGreca no. 1!), also deposited in CUP, was identified by Dick: *Sarcosphaera gigantea* (Rehm) Kanouse. I collected it in 1989 after completing Dick’s Field Mycology course. It was a hot summer day when I brought the specimen to him for identification, and I was shocked to see him shirtless in his lab: seeing my formal professor in such an informal situation was strange to me! But I quickly got over my surprise when he excitedly took the fungi from my collecting bag and started poring over his identification books, quickly making a slide to examine its spores. Later, he also showed me how to make the cups “poof” their spores. I’ll never forget that day.

Right up until his death, Dick’s collaborations with other scientists around the world inspired activity and growth for us here at CUP. For example: there was a mycology student in the Canary Islands (Luis Quijada) who had just completed his PhD in the systematics and floristics of cup fungi on the Canary Islands. Dick ran an NSF-funded project on Canary Islands cup fungi for years (resulting in many publications) and so Luis emailed Dick with some questions, which led in turn to his requesting multiple loans of Canary Islands specimens from us, which allowed (a) completion of his dissertation and (b) improvement of our specimens here at Cornell with Luis’s examinations/annotations.

Another example: Madame Françoise Candoussau, a long-time friend of Dick’s in France, has just this year decided to give her valuable personal herbarium (comprising thousands of specimens, mostly from Europe) to Cornell as a direct result of her friendship and longtime professional association with Dick Korf.

I should also mention that Dr. Korf was innovative in his teaching. At a time when team-teaching was almost unheard of, he divided the work of his Introductory Mycology class, inviting specialists of different groups of fungi to teach for different weeks. Dick himself taught the first half of the course, while other, 'guest' teachers took over the last half of the course. This allowed for a more thorough mycology education for his grateful students. I know this sort of thing is commonplace now, but at the time (25 years ago—1991) this was a rare thing.

After I left Cornell and came back to work here with a PhD in lichenology, I joked with Dick that he'd inadvertently turned me into a lichenologist because he always dismissed any lichen specimens that students (including me) brought him. "Lichen!" is all he would say, tossing the specimen aside. I found it fascinating that such a great mycologist had such a poor opinion of (and lack of interest in) lichenized fungi, and this spurred me on to learn more. So in an indirect way, I credit Dick for inspiring my career as a lichenologist.

—SCOTT LAGRECA, Curator

Cornell Plant Pathology Herbarium, Ithaca

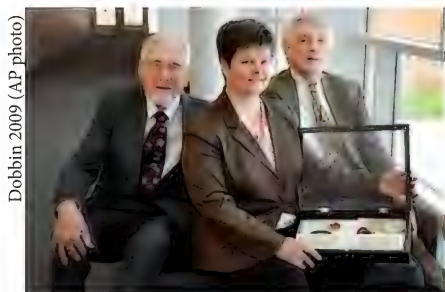
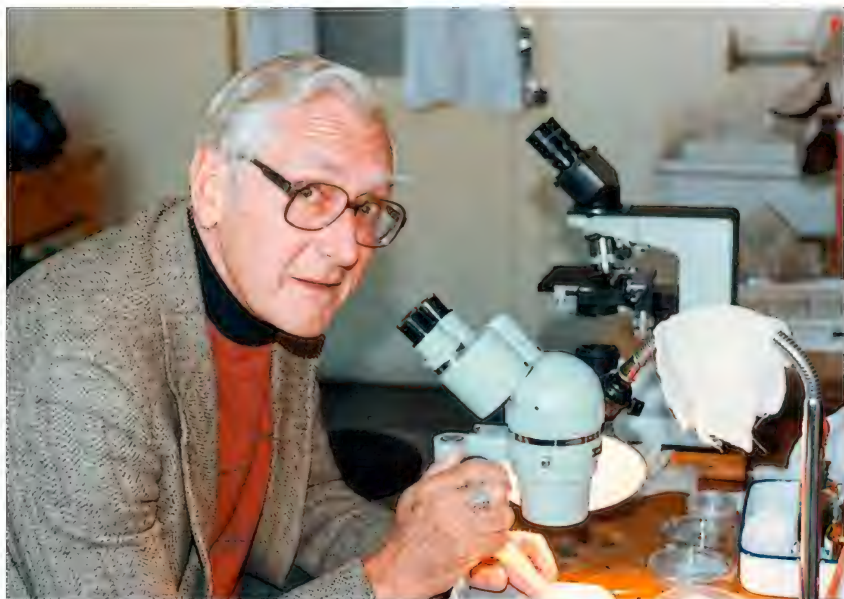


FIG. 15—Professor Emeritus Richard P. Korf, CUP Director Kathie Hodge, and Curator Robert Dirig pose with some specimens from Cornell's Fungi of China collection in 2009.

From China to CUP and back again

"Shu Chun Teng traveled halfway around the world on a scholarship to study mycology at Cornell University in 1923. He left five years later with a knowledge of fungi unequaled in China, then spent the next decade traveling on horseback gathering up molds, lichens, yeasts, rusts and morels in the forests, fields and marshes of his homeland. ...During the Japanese invasion in 1937, Teng arranged for his best specimens to be removed from a national botany institute he directed in Nanking to save them from destruction. During World War II, they were smuggled by ox cart to Indochina and then by sea to the United States, and 2,278 of the specimen packets ended up at Teng's alma mater.

"At Cornell's initiative, the university is dividing up and sharing its Fungi of China Collection with the Academy of Sciences in Beijing to help advance the



Wen-yen Zhuang; Courtesy of IMA Fungus 2016

FIG. 16—Dick at the microscope in 1988 at the
Key Laboratory for Systematics & Microbiology in Beijing.

exploration of fungal species. ...In a repatriation ceremony Monday, Cornell President David Skorton presented a high-level Chinese delegation with a rare mushroom called *Lentinus tigrinus*, reaffirming the university's desire to share a collection he said it 'has held in safekeeping for the global scientific community since 1940.' ...Some 1,700 specimens will be delivered to China in the fall, including 57 considered irreplaceable. Cornell will retain fungi that can't be divided, but make them available to scholars.

"...At the start of the devastating Cultural Revolution in the 1960s, Teng was tagged as a 'counterrevolutionary academic authority.' Discharged from his lab, he was subjected to daily beatings and mental persecution that ruined his health and career, according to the science academy. He died in 1970 at age 67. Years later, his family managed to recover Teng's confiscated manuscripts. His daughter, Rosaline Deng, worked with a Cornell professor of mycology, Richard Korf, to complete and publish *THE FUNGI OF CHINA* in 1996 (Teng 1996).

"Transferring the fungi 'is our decision,' said the 83-year-old Korf, who taught at Cornell until 1998. 'The whole reason really is that it makes scientific sense, especially with the warming of U.S.-China ties over the last 30 years. ...It's clear we are cooperating in ways we never knew we could.'

“Another impulse ... is I had a brilliant student, Wen-Ying Zhuang, come to work with me from China in the 1980s and get her Ph.D. She became a major figure in the Chinese mycological field. She certainly pressed me on how important it would be for Chinese scientists to be able to easily access those specimens.”

—BEN DOBBIN, Associated Press (Dobbin 2009)



FIG. 17—Seeing the sights on the way home
from the 1986 MSA Annual Meeting in Gainesville, Florida.

Students and Fellow Travelers

“Dick obviously enjoyed his students, believed in them, and wanted them to be successful. As such he encouraged them to attend scientific meetings doing whatever he could to ensure that they managed to get there. This included towing his own pop-up camper staying with his students in a campground near the meeting site.” —Rossman & Zhuang (2016)

“One of the world’s most notable mycologists of all times died on August 20, after ninety-one years of a well-lived life.

“Dick Korf, Professor at the Plant Pathology Department at Cornell University, was a leading figure in the study of discomycetes (*Leotiomyces* and *Pezizomyces*) and a mentor to many in this field. Not only did he convey his love and appreciation of fungi but also taught us the importance of rigorously studying the morphology of fungi and their role in nature and the environment.

This came with hours of hours of observations of each specimen under the microscope, methodically recording morphological and anatomical features and chemical reactions. He always brought an inquisitive mind, teaching us to formulate questions, to doubt dogma and try to answer questions with rigor. At the same time he taught us the intricacies of nomenclature, again with rigor.

"The first issue of MYCOTAXON, a journal founded by him, dedicated to the taxonomy and nomenclature of fungi, came out in 1974. It is today in its volume 131 and has been a leading journal for descriptive mycology.

"Dick was an advocate for social justice, cared for others, and helped many in need.

"Today is a sad day for his family, to whom he was completely devoted. It is a sad day for us, his students, and for the many that he mentored, advised, and helped through his well-lived life as a caring person and as an eminent mycologist."

— TERESA ITURRIAGA

MSc (1983), PhD student (1990) Cornell, under R.P. Korf



FIG. 18 (LEFT)—Dick, Linda Kohn (student & future 1999–2000 MSA president), Trond Schumacher (future 2000–2006 IMA president), and Japanese colleague in 1983 at the Sanshi Imai Memorial Discomycete Foray after IMC3 in Nikko, Japan. FIG. 19 (RIGHT)—Kumi and Dick at Satoshi and Harumi Inoue's wedding at Cornell's Stadler Hotel on September 25, 1995

"When I entered Department of Plant Pathology of Cornell University as a graduate student in 1990, Dick was one of a few professors I met during my orientation days. I had graduated from Tohoku University in Sendai in 1981 with Agronomy background and didn't know much about Mycology. Our conversation was not so much at that time yet.

"I started my M.S. degree with Dr. Jim Aist and selected Mycology as my major. One for that decision was that I did not want to take a course "SADISTICS" (statistics).

"I ended up taking almost of all the courses offered by Dick during my M.S. degree period. One of the toughest courses that I have ever taken is Advanced Mycology (PL PA 739) during the 1992 fall semester. The TA for the class was Julie Carroll and my classmates were Linda Hanson, Ker-Chun Kuo, and Kathie

Hodge (who is currently teaching Mycology at Cornell). Dick gave us a very strange project and we struggled to collect and identify ten (10) uncommon species of hyphomycetes.

“I obtained my M.S. degree and entered my Ph.D. program in 1993. In 1995, just after I passed my A-exam, I married Harumi. We moved to Dick and Kumi Korf’s apartment and lived there for two years until I obtained my Ph.D. and returned to Japan. Our son, Amane, was born there in 1996. We spent a very happy time with Dick’s family and have beautiful memories of them. I remember it as if it were just yesterday.

“With my deepest sympathies on the death of Dick”

SATOSHI INOUE, PhD (1997), Cornell under R.P. Korf
Director of Forage Seed Department, Kaneko Seeds Co. Ltd., Japan

“When I was a senior at Colgate, I was assigned the task of “refreshing” cultures of *Rhizopus nigricans* to restore their ability to form zygospores. Knowing nothing about a process to accomplish this, I wrote to Korf at Cornell, who kindly sent me two new cultures and a method to demonstrate zygospore development.

“And so it was that I applied to Cornell and entered in Fall 1956. My intention was to perform taxonomy on jelly fungi, but I suffered a self-inflicted wound when I skipped numerous labs in Plant Path 1 and earned a C in the course, which violated Cornell’s dictum of grades of Bs or better for continued enrollment. I was informed that while I might eke out a master’s degree, I surely would not be allowed to continue thereafter.

“Thus it was that I enrolled at Columbia University under Lindsay Olive. And the rest is history. In one of my rare subsequent meetings with Dick Korf, he referred to me as “the one that got away.”

“Although Dick was not high on the marquee in his last years, his name will surface for generations as the co-founder of MYCOTAXON, discomycete taxonomist, and outstanding personality in the mycological community.”

—RON PETERSEN. Professor Emeritus & MSA President (1993–1994)
Ecology & Evolutionary Biology, University of Tennessee

“Dick Korf was instrumental in my existence as a mycologist. In 1970 I was a floundering second year graduate student in mycology with no real idea of a research project or direction. Dick wrote to my major professor, Bill Denison (a student of Dick’s) at Oregon State University, inviting him on a collecting trip in Puerto Rico and Dominica. Bill couldn’t go, but I jumped at the chance. That trip was magic, and I met my lifelong friend Don Pfister. During the day we groveled in the litter finding tiny discomycetes as well as heaps of delightful yellow and red perithecia and black, twig-like xylarias. In the evenings we crowded around the microscope attempting to identify them at least to genus.



Wen-yen Zhuang, courtesy of IMA Fungus 2010

FIG. 20—Newly installed MSA President Amy Rossman with Richard ‘Elias Fries’ Korf at IMCV, Vancouver, BC (Canada) in 1994.

Later I learned, at least for the nectria-like fungi, that, no, I wasn’t stupid, there really were no keys to tropical species, and that was the inspiration for research on these fungi.

“The home of Dick and his wife Kumi was always open to itinerant mycologists even when their four children were small. Although chaotic during the day, Dick would rise early in the morning to work on MYCOTAXON in those quiet hours. He was totally supportive of graduate students and postdocs writing glowing letters of recommendations—perhaps tapping into his creative writing tendencies! But they worked, as most of us found good jobs in the always-tough mycological employment marketplace. He shared his time and resources, always willing to stop his own work to discuss nomenclature issues or read over a draft manuscript.”

— AMY Y. ROSSMAN
Oregon State University, Corvallis



Amy Rossman (IMA Fungus 2016)

FIG. 21—Kumi & Dick at Amy Rossman’s home in Beltsville in 2000.

FIG. 22—At Amy’s in 2005: mmmm ... roast QUORN!

Fungal taxonomy and nomenclature goes international

These messages and reminiscences are organized alphabetically by country.

VALE DICK KORF

Sad news about the passing of Dick Korf

Among his numerous contributions to mycology, Dick Korf was Secretary of the NCF (then the Committee for Fungi and Lichens) in the 1980s and co-founder and long-time supporter of MYCOTAXON.

Two enduring images are early career Korf at the blackboard (note the peace sign belt buckle in FIG. 12): and later, channeling Elias Fries.

— TOM W. MAY, Secretary, IBC Nomenclature Committee for Fungi
Royal Botanic Gardens Victoria, Melbourne, Australia

We regret so much for Dick Korf.

We'll always remember him for his great mycological contributions and inspiration.

— FRANCISCO CALAÇA
Lab. de Biodiversidade do Cerrado - Fungos Coprófilos
Unidade Universitária de Ciências Exatas e Tecnológica, Anápolis, Brazil

Dick's passing marks the end of an incredible career and great loss for mycology. I thank him for his friendship and kindly guidance and send my sincerest condolences to his family and friends.

—SCOTT REDHEAD, Curator, DAOM
Agriculture & Agri-food Canada, Ottawa

Dick began his association with the Institute of Microbiology (Chinese Academy of Sciences) in Beijing and had worked hard to promote Chinese mycology, especially through publishing major books on fungi through MYCOTAXON (IMA 2013).

In China, "an 88th birthday is regarded as particularly special, and those that reach it are called "Mi-shou". In honour Dick's attainment of "Mi-shou" status, the June 2013 issue of MYCOSYSTEMA (on whose editorial board Dick served since its founding in 1987) was dedicated to him (IMA 2013). The excellent photos of Dick's 1988



Wen-ying Zhuang (IMA Fungus 2016)

FIG. 23—Dick at the Jinzhong temple in Yunnan, China (1988)



Korf in China in 1998. FIG. 24 (left): Before Tanzhesi temple in Beijing.
FIG. 25 (right): searching lily pads at the Xishuangbanna Tropical Botanical Gardens.

visit to China (FIGS 23–25) were captured by Wen-ying, his student and much-valued colleague and close friend.

—WEN-YING ZHUANG,

Key Laboratory of Systematic Mycology & Lichenology
Institute of Microbiology, Chinese Academy of Sciences, Beijing, China



FIG. 26—The discomycete team at IMC7 outside the Oslo convention center in 2002.



Courtesy of Rafael F. Castañeda-Ruiz

FIG. 27—Marc Stadler (Germany), José Guarro (Spain), Teresa Iturriaga (Venezuela), Dick (USA), and Rafael Castañeda-Ruiz (Cuba) represent three continents at the 2011 Latin American Mycological Congress in Costa Rica.

I have received the terrible news from Teresa Iturriaga. Please pass my condolences to the family of Richard Korf and share this photo (FIG. 27) of Dick with friends at the 2011 Latin American Mycological congress in Costa Rica.
Un abrazo, Rafael

—RAFAEL F. CASTAÑEDA-RUÍZ
Fundamentales en Agricultura Tropical “Alejandro de Humboldt”,
Santiago de Las Vegas, Cuba

It has just come to my attention that Richard Korf, co-founder of MYCOTAXON, has passed away on Aug. 20th. Please accept my sincere condolences for an outstanding mycologist and a visionary who will undoubtedly be remembered as one of the all-time greats in mycology.

—MICHAEL LOIZIDES
Cyprus Mycological Association, Limassol, Cyprus

Nobody like Dr. Korf, so professional, so enthusiastic of small discomycetes, but most of all, a good person. We will never forget him.

—ROSARIO MEDEL ORTIZ, Instituto de Investigaciones Forestales
Universidad Veracruzana, Vera Cruz, Mexico

Dear Dick

I first met you when you visited me when I was working in the University of Hong Kong in the mid nineties. You were so generous and supportive of my work at the time and gave me encouragement to continue my efforts

The next time I met you was at the International Mycological Congress in Norway. You found it so funny that I did not recognize you dressed up as that ‘other’ famous scientist.

Since then you have always been so supportive and provided advice and encouragement.

I will dearly miss you.

—KEVIN DAVID HYDE
Editor-in-Chief, FUNGAL DIVERSITY
Director, Center of Excellence in Fungal Research
Mae Fah Luang University, Chiang Rai, Thailand

Was so sad to learn of the passing of Dick. He was hugely influential in mycology. Truly one of the greats of the 20th century.

—DAVID W. MINTER

President, International Society of Fungal Conservation
Egham, Surrey U.K.

Dick was inspirational and I shall make sure that the BMS office knows. I had a lovely dinner with him in Louvain in the late 1990's and I certainly valued his help, advice, and support.

—ANTHONY J.S. WHALLEY

School of Biomolecular Sciences
John Moores University, Liverpool England U.K.

I first met Dick at IMC1 at Exeter (UK) in 1971. I had joined the Commonwealth Mycological Institute two years before, and this was my first exposure to the international mycological community. I especially remember a discussion on nomenclature he chaired, and which led to the establishment of a Nomenclature Secretariat under the auspices of the IMA. He was especially concerned about the problems due to the later starting point for names of non-lichenized fungi, including publishing a series of papers he characteristically entitled 'Later starting point blues;' the rules were changed in 1981. As Secretary of the Nomenclature Committee for Fungi, he played a major role in debates at International, Botanical Congresses, representing mycological interests, where his acting skills were often put to good effect.

His love of fungi and fieldwork inspired not only his many graduate students, but also many others throughout the world, especially tropical areas still so little known for discomycetes. I was pleased to recognize this a little when introducing *Korfiomyces* (along with Teresa Iturriaga) for a little disco from Venezuela that had puzzled him. Perhaps the most important thing I learned from my contacts with Dick was the importance of always being fully aware of the historical background of issues and thoroughly researching the consequences of any contemplated actions. We did not always agree on controversial matters, but always respected each other's views.

His foresight in establishing MYCOTAXON to address a then developing log-jam in getting systematic mycology papers in print, with the innovative requirement for authors to produce their own camera-ready copy, was an enormous stimulus to the health of the subject. He was a most generous man, and kindly 'loaned' me a set of the printed volumes after I left the International Mycological Institute at the end of 1997. The neat rows of brightly coloured volumes, which I repeatedly have to consult, are an incredible legacy.

Dick was always keen to promote mycology selflessly throughout the world, not least in China where he helped safeguard collections, get several major

works published, and was one of a small group ‘foreign’ mycologists involved in the establishment of MYCOSYTEMA prepared by the Chinese Academy of Sciences, which has now also grown into a major journal in the region.

An inspirational mycologist who touched the lives of numerous mycologists around the world, many of whom came to occupy significant positions. The carefully executed works from their ongoing research—and of those they in turn train and inspire—that have the stamp of Dick’s concern for accuracy and quality will be a self-perpetuating memorial. I feel privileged to have known him, and would have loved to have been able to spend more time benefitting from his vast experience and insights.

—DAVID L. HAWKSWORTH

Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, U.K. & Department of Life Sciences, Natural History Museum, London, U.K.

FIG. 28—Dick, wearing his ‘Reunite Gondwanaland’ t-shirt, makes a fine point at the 2003 New England Mycological Foray (NEMF) in Deposit, NY. Of the 24 species he identified, five were new to the 27-year old NEMF master list.



Diane Smith; NEMF foray website

My first acquaintance with Dick Korf was as a new member of Mycological Society of America in the early 1960’s. He and Clark Rogerson literally WERE the MSA. He had an imperious personage—handsome, self-confident, theatrical, and well spoken. He seemed initially remote from young mycologists, but accepted them as colleagues over time. I was flattered when he asked me to be a founding member of the editorial board of MYCOTAXON, a position that I enjoyed for many years.

Dick was a consummate teacher of mycologists. He took them on field trips to places that seemed exotic to a small-town man. His former students are among the leaders of mycology—Jim Kimbrough and Don Pfister among many others. He was also a teacher at MSA meetings—quizzing students and professionals on details of their studies and offering advice on improvements.

It was as perhaps nomenclaturist that he was most widely known. He spent much of his time solving nomenclatorial problems for colleagues worldwide. He was dismayed with massive changes in the mycological part of CODE OF BOTANICAL NOMENCLATURE and I suspect that, had he been younger and

healthier, that he would have used his vast intellect and assertiveness to prevent the most irrational changes.

Let us praise this great scholar! Mycology is poorer without him and we shall never see his like again.

— JACK ROGERS,

Professor Emeritus & (1977–1978) MSA President
Washington State University, Pullman, WA U.S.A.

The Lion in Winter

Except for a 1950–1951 tour as Lecturer at Scotland's University of Glasgow, Dick devoted his entire research life to Cornell. His heart, however, belonged to his family, and an impressive one it is. In its praise of "Hidden Books: the art of Kumi Korf" (2011), the National Museum of Women in the Arts refers to Kumi as "one of the most original artists in the United States." Works by Dick's wife have been exhibited worldwide and (of the 25 collections cited) include the Library of Congress, Harvard's Houghton Library, the New York Public Library, London's Victoria and Albert Museum and Tate Library, Hungary's Savaria Múzeum, and the Cleveland Museum of Art. He and Kumi are the proud parents of Mia (an American film and television actress), Ian (Associate Director of Bioinformatics at UC Davis), Ian's twin Mario (Principal Technical Writer at Akamai Technologies), and Noni (Director of Learning Innovation & Design at University of Michigan in Ann Arbor). On his retirement, Dick obviously basked in the glow of his family's creative energies.

"During his stay at the Yokohama National University (1957–58) as a Fulbright fellow, Dick met and



Courtesy of Pavel Lizon



Mia Korf; reprinted from Wilensky 2013

FIG. 29 (above). Dick & Kumi at home in Ithaca with Pavel in 2005. FIG. 30 (below). Dick relaxing during the summer of 2013.

married Kumiko Tachibana. Kumi graduated in architecture and later print-making at Cornell. She is very active as a visual artist to this day.” (Lizoň 2016)



Courtesy of Pavel Lizoň

FIG. 31—1993–1998 MYCOTAXON E-i-C & close friend Lizoň chats with Dick in Ithaca in 2005.

“Exe Island on Big Rideau Lake in Canada was purchased by the Korfs in 1972 (later transferred to Mycotaxon Ltd as Exe Island Biological Station). The simple cottage and 3-acre island became a favourite holiday destination for their four children and friends.

From my own experience I must say that it is a great place for swimming and diving, sailing and waterskiing, fishing, barbecuing, or just having a good time. Dick organized famous Crazy Eights card tournaments not only on the island but also during several mycological meetings and everyone who has participated remembers having a lot of fun.” (Lizoň 2016)

Cover design by Noni Korf

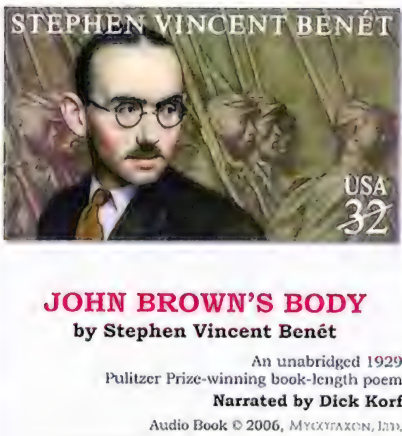


FIG. 32—Dick’s tour de force at age 80.

In mid-2007, an audio gem from Dick arrived in the editorial mailbox: his 12-disc audio recording of the epic poem, JOHN BROWN’S BODY. Next fall’s solo 1500-mile road trip transformed a welcome gift into one of my most valued possessions. Dick’s voice so mesmerized me during my Montana journey to visit my father that I listened to it all over again on my return to Oregon. This tour-de-force grips from the first disc and never bores. The text below has been ‘cribbed’ from Dick’s liner notes and a blog by John Brown’s great-great-great-granddaughter.

"This recording was made in 2006, a last gasp of an 80-year-old lifelong actor, the culmination of a 20-year dream. It is dedicated to my actress daughter, Mia Korf, for encouragement, and to my wife, Kumi, for everything.

"I grew up as a ravenous reader, encountering Stephen Vincent Benét's *John Brown's Body* at the age of 14, I was captivated by the book, which I read and reread over the ensuing sixty-some years. It surely helped form me into an anti-war activist...

"Poetry has a very special place in my heart, and as a youth I began reading and writing poetry. I agree with Stephen Vincent Benét: poetry begs to be read aloud. The skilled poet may embed in his poems frequent 'stage directions' in the choice of typographic tools (punctuation, the use of parentheses, italic typeface, paragraphs, long dashes, indentations), and of course changes in meter or rhyme. Benét's use of these tools simplified my narration of the poem; these are treated here as not only readers' but narrator's guidelines." (Korf 2006)

"THURSDAY TREASURE - RECORDING OF JOHN BROWN'S BODY" (McCoy 2009):

"Over the past 30 years, I have been the recipient of some fascinating letters, emails, FaceBook notifications, and phone calls from people regarding John Brown. One such FaceBook connection occurred in June of this year and resulted in today's featured treasure. Emeritus Professor of Mycology at Cornell University, Richard P. Korf wrote me the following:

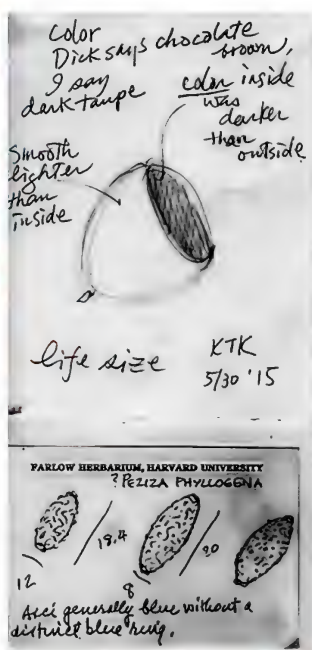
"I assume you are the lady I read about in an article sent to me by my cousin from her local paper, indicating that you are a great-great-great-granddaughter of John Brown of Harpers Ferry fame. I have a strong connection, in that I was very influenced by the story, and particularly the book-length poem called *John Brown's Body* by Stephen Vincent Benét, which received the Pulitzer Prize in 1929. Probably you have read it. Oddly enough it never was made into an audio book. I finally discovered that there is a copyright issue, and managed to get permission to do a "not for sale" version (I am now 84, and a lifetime actor) which I recorded in 2006. I have a few copies left and would be happy to send you one (it is on 12 CD discs, 13-1/2 hours long!). You can send me your postal address if so."

"Think about this, Professor Korf was so enthralled with *John Brown's Body* by Stephen Vincent Benét, that he spent considerable time, effort, and his own money to manufacture 100 limited editions of a 12 CD set that he cannot sell due to copyright regulations.

"I immediately responded that I would be honored to receive one of his recordings. A few days later I received a package in the mail with a professionally packaged, produced set of CDs. Professor Korf's voice is a joy to listen to, and the set is amazing. The front cover of the box features the Stephen Benét

postage stamp, while the back is the 1859 photograph of John Brown with his full beard. Inside each CD is labeled and tucked into its own pocket. ...A truly impressive presentation. It is a shame that due to copyright legalities, Professor Korf is not permitted to offer his incredibly moving rendition of this important work of literature, but I am truly blessed to have received a copy from him. I am holding onto this treasure. When I walk past my bookshelves and see the case, I always think fondly of the Professor whom I have never met, yet he felt inclined to share his lifelong dream with me.” (McCoy 2009)

Kumi's mystery fungus, found at home in August 2015. FIG. 33 (LEFT): Tiny red cups delight Teresa & Dick; FIG. 34 (RIGHT): The sketch by Kumi with Don Pfister's annotations added; FIG. 35 (BOTTOM): Dick supervises Teresa's note-taking skills.



KUMI'S MYSTERY FUNGUS—Here are some photos (FIGS 33–37) of my last visit to Dick in August 2015 when he described and made me take very detailed notes on 'Kumi's fungus'.. while he described in detail the most impressive manner how this fungus shattered. When I returned to the Farlow with the specimen, Don [Pfister] immediately looked at it under the microscope, made drawings of the spores (also attached), and identified it to *Peziza phyllogena*, which Korf himself had worked on.

On that visit he impressed me once more with his absolutely keen memory and sense of humor when he told us the story of Schulzer von Muggenbruck, who described twice as new (and from the same specimen) *Peziza heterotropha* Schulzer 1878 and later as a new genus and species, *Strossmayeria rackii* Schulzer 1881 [now listed as *S. basitricha* (Sacc.) Dennis on www.indexfungorum.org].



The holotype of the name of *S. rackii*, along with most of the Schulzer Herbarium, was lost, so Dick decided to undertake an expedition to find a neotype for this fungus and two other type specimens needed to solve some other taxonomic issues with other groups, which we described in detail (Korf & al. 1988).

During such expeditions to collect discomycetes, he taught us how to find these organisms: swimming among leaf litter, staring at little branches, twigs, leaf blades, leaf petioles, in a place where at first nothing could be seen, and as he stated “they’ll start winking at you”... and so they did!

Each of those collections was carefully removed from its substrate with a knife—at lunch time he would love to let us know he was opening the only sardine can with the “dung knife”—and placed in a glassine envelope. Rigorous notes were taken of textures, shapes, colors and sizes, and then the magic of examining the microscopic structures started. Looking at the wonders of ascospores, septa, ornamentation, crystals, colors, and the Melzer’s reaction took us always late into the night. Sleep was of no concern to Dick when there was so much fun looking at these amazing structures!

And by the way, we did make 50 collections of *Strossmayeria rackii* and thus were able to neotypify this genus.

—TERESA ITURRIAGA

Data Curator, Microfungi Digitization Project, University of Illinois



FIG. 36—Teresita and Dick,
August 2015

In a subsequent message, Teresita referred to a Vladimir Nabokov poem that so enchanted Dick that he pasted it onto the arm of his microscope. It is only fitting that her fond reminiscence of her friend and former professor conclude with the stanzas from the Russian author & lepidopterist’s “On Discovering a Butterfly” that convey the passion of all those longing to reveal Mother Nature’s hidden framework.

Smoothly a screw is turned; out of the mist
two ambered hooks symmetrically slope,
or scales like battledores of amethyst
cross the charmed circle of the microscope.

I found it and I named it, being versed
in taxonomic Latin; thus became
godfather to an insect and its first
describer—and I want no other fame.

—Vladimir Nabokov (1943)



FIG. 37—Dick in the heart of his wife, children, and grandchildren during the annual gathering of the clan (Summer of 2016).

A daughter's farewell

My dad's passing means that there are many untold stories that can no longer be told. Many photographs that can no longer be identified. He was preceded in death by his older brother and his cousin and, at 91, by many his friends, colleagues, and students. I would have liked to hear more stories about his childhood, because looking back there are so many narrative threads that could be followed to their source.

I do know that the publishing of the journal, MYCOTAXON, was not his first adventure in publishing. As a child he had a small letterpress at which he set type, printing up a local gossip rag, some stories surely fabricated, which he then sold to his neighbors. It was a novel and entrepreneurial undertaking, quite unlike MYCOTAXON but the connection is there.

I remember the genesis of the journal, and the way that my father's eye would light up as he would describe the concept—a journal which allowed authors to publish quickly and for free, that used offset printing technology to shortcut more time intensive and expensive methods of printing, where the journal wasn't responsible for the review process, and where volumes would be issued when they reached a certain number of pages rather than a certain time period. I don't know how novel the journal's "find your own reviewer" peer-review process was, but he told me he heard many stories of authors who were able to get peer-reviews from people that they highly respected and to whom they would not otherwise have dared to communicate.

Then there was all the stuff. We sold special paper that he designed that had two sets of lines margins laid out in light blue ink so that authors could type onto this paper, using either an elite or pica typewriter, and then mail in camera-ready originals. We also sold, and used ourselves, special Letraset dry transfers for numbering photos and creating somewhat consistent arrows, page headers, and other typography. We would receive back from the printer rolls and rolls of acetate which were cut up for reprints, sometimes mailed back to authors if they requested it for them to make their own. And there were so many different sizes of envelopes and boxes for shipping the journal out.

When an issue was published, there would be a stack of some 400 or so brightly colored journals ready to be sent out. We tried many different ways of printing up labels for these and back then we had to sort them all by zip code



FIG. 38—Noni designed this logo years ago for one piece of 'stuff'—the official MYCOTAXON t-shirt worn proudly by journal disciples. With our move to online publication in 2011, Noni modified the logo for use on our back covers, beginning with MYCOTAXON 115. Dick approved!



Kumi Korf; courtesy of Teresa Iturriaga

FIG. 39—Noni, Dick, and Teresita share a happy moment in August 2015

and country before dropping them at the post office. We made stuffing the envelopes into a game, quizzing each other on the zip codes of certain cities, or the middle names of our subscribers. I knew many, many names and addresses of mycologists by heart, learned of libraries from all over the world, imagined the journal traveling there to be opened by someone who must have wondered why the journals they received were never the same color. It was a game, it was a chore, it was something we all chipped in on 4 times a year. One time I slipped a message into the envelope—it was being shipped to Linda Kohn—and I wrote “help, I’m trapped in a cottage industry!”

Every now and then someone would order a backorder or two, or sometimes even a whole run, and this was a cause for excitement, and more boxing, and M-bags, and international postage. I sometimes thought that the post office was my father’s home away from home. He was meticulous in understanding the rules and could read the charts and scales as well as any postmaster. He always tried to stamp the envelopes with the most exotic and fanciful of US postage, in case the recipient was a stamp collector. He had many difficulties with the post office, however, because the journal didn’t fit into a certain category of a quarterly publication, so we didn’t qualify for 3rd class mail, and ultimately the crushing cost of postage was one of the reasons we found we had to move to publishing online.

As I got older I learned to work on the subscriptions management. At the time this meant gathering checks, stamping them “for deposit only” and entering the payments on 3x5 inch index cards. Some people were individual

subscribers, some were institutional, some we invoiced, some had standing orders, some were organized through companies such as Ebsco. It was all very detailed and precise, and hand written. Once I started doing the subscriptions management I became even better at the box stuffing game of “in what city does mycologist X live?” As this modernized, the job included running credit card slips through the embosser and mailing back receipts. Eventually there were uploads via a dial-up modem. And there were also the odd international money orders, which my father would have to cash at his home away from home, the post office. So many memories of these little details and all the things it took to keep the journal running.

At some point my father embraced the computer, bought a Mac IIe, and began getting fascinated with the computer as both a administrative tool and one useful for authors. I think we were all very tired of typing on that special light-blue lined paper, and needing to use whiteout to make corrections. The early volumes do have the DIY look of a zine, but the computer made submissions of articles and reviewer notes much easier for the author, but also added to the work of the editor. There’s no doubt my father loved working at the computer, teaching himself new software, designing forms and databases to track subscriptions, and authoring articles. I can see him in my mind’s eye, hunched over the keyboard, using only two fingers to hunt and peck at a maniacal speed as he corrected a manuscript, or communicated with a former student.

The printed word was very important to my father. He was a keen critic of his own writing as well as that of others, helped many people to edit their manuscript and was always on the lookout for readable prose and the proper use of the English language. He would admonish writers to “kill your darlings” and when we were developing the Mycotaxon website, pointed out that I had failed to italicize a period. Before my father died we had a project to pull together all of his writings and bind them together. I regret I wasn’t able to complete the project, the reprints were of so many different sizes I couldn’t figure out how to put them together. But together we marveled at the stack of pages. Over 400 different publications! I so wish I could have also added to the stack his first published articles, the 1935 gossip rag from White Plains, NY.

I miss my dad, and know he would have edited the hell out of this piece.

NONI KORF
11 January 2017

Ode to RPK

Awake, my Muse, and let us chant again,
for Richard has attained four score and ten,
A life well lived, and never could my song
number the landmarks of a life so long,
so rich, so varied, put to so good use,
for like that busy man, Odysseus,
patient, resourceful, skilled in many arts,
our Richard is a man of many parts:
In lusty youth he journeyed to Japan,
and bore away the fair Kumikosan,
whose beauty lives undimmed, though made to bear
sturdy twin sons, and daughters passing fair.
Master of mushroom lore, taxonomist,
shepherd of mushroom hunters, lengthy list,
whose fungal gleanings will survive the ages
in MYCOTAXON's printer-ready pages.
A scientist, a teacher wise and stern,
his life will sometimes take a different turn;
he is no stranger to the sacred rage,
that brings forth sound and fury on the stage;
sailor intrepid, always northward bound,
until one happy day he ran aground,
and entered on the hero's life we know,
lord of the wooded isle in Big Rideau.
There let us leave him, Muse, for you grow faint;
a full length portrait genius alone could paint.
Let's be content to let our rhyming cease,
a gift of love, though not a masterpiece,
a song with but a simple tale to tell:
Richard, we honor you, and love you well.

—Peter Wetherbee
in honor of Dick's 90th birthday, 2015

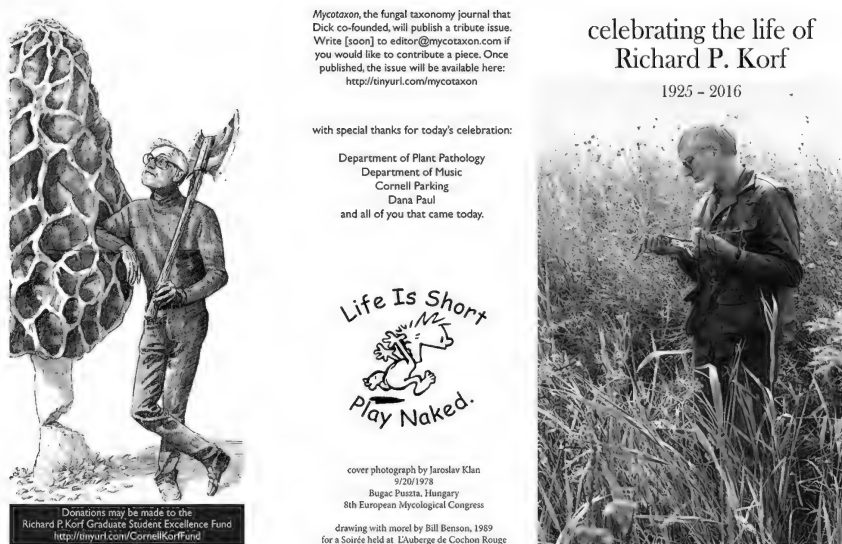


FIG. 40—On December 18, 2016, the Korf family hosted a celebration of Dick's Life at Cornell. The back of the program above provided the liner notes from Dick's audio book version of John Brown's Body and his obituary, featuring the 1972 photo of Dick at the Plant Path lab blackboard. Scheduled speakers included daughters Noni & Mia Korf, students Don Pfister & Kathie Hodge, sons Mario & Ian Korf, and grand-daughter Maïa Korf followed by memories shared at an open mic during the reception on stage. Not surprisingly, festivities continued well into the evening at Noni's place.

Acknowledgments

Many have contributed to this celebration, but special editorial thanks are due Noni Korf, who has borne much during the past year yet agreed to serve as family liaison and scribe in addition to MYCOTAXON web-master while holding down a fulltime new job. We both thank everyone who shared memories and photos in this memoriam: Francisco Calaça, Rafael Castañeda, David Hawksworth, Kevin Hyde, Satoshi Inoue, Teresita Iturriaga, Michael Loizides Hannes Maddens, Scott LaGreca, Pavel Lizoň, Tom May, Rosario Medel, David Miner, Ron Petersen, Scott Redhead, Jack Rogers, Amy Rossman, Tony Whalley, and Wen-ying Zhuang. We also thank Pedro Crous, David Hawksworth, Manon van den Hoeven-Verweij, Pavel Lizoň, Amy Rossman, and Wen-ying Zhuang for granting permission to reprint excerpts from IMA FUNGUS.

The Editor-in-Chief, who assumes sole responsibility for shamelessly purloining reminiscences and photographs from the Internet, is especially grateful to Kathie Hodge and Scott LaGreca for providing such entertaining and informative blogs about Cornell. The photograph of Elias Fries downloaded from the MUSHROOM, THE JOURNAL website (www.mushroomthejournal.com) is elsewhere listed as in being the public domain. Other attributions are placed next to the photos, presented in the text above, or covered in the references cited.



FIG. 41. Watkins Glen State Park, NY

Excuse me, while I disappear.

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Jafar Shokrollah Zadeh

MEENA, CHANNELING DICK
(2016)